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

iSBTC 10-13 November 2005
Valeria Milani, MD, PhD
Munich

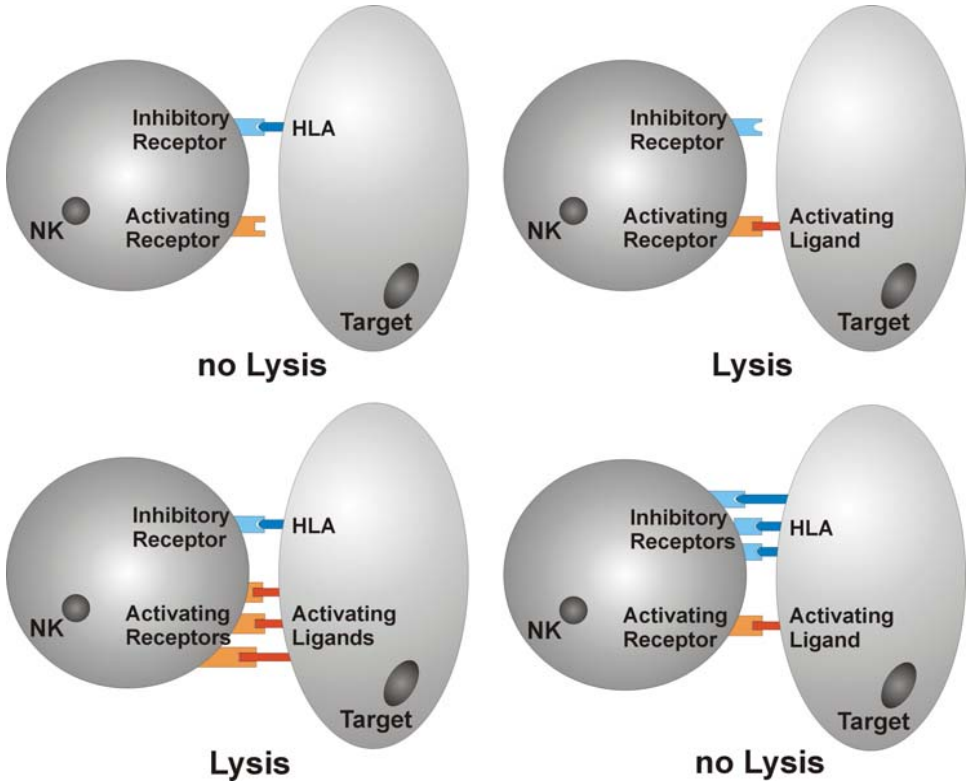
Agenda

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

1. NK ligands

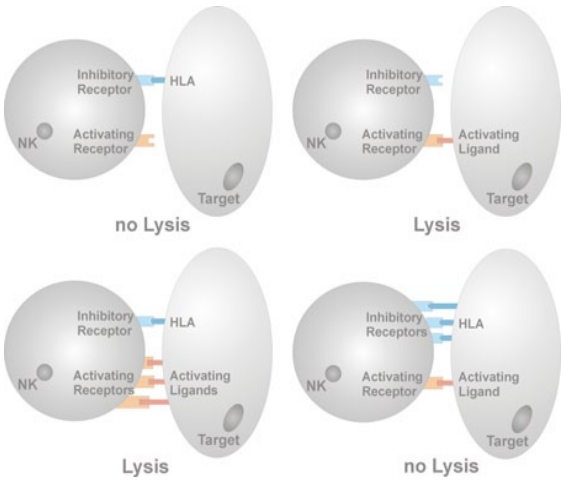
NK ligands

Missing self hypothesis

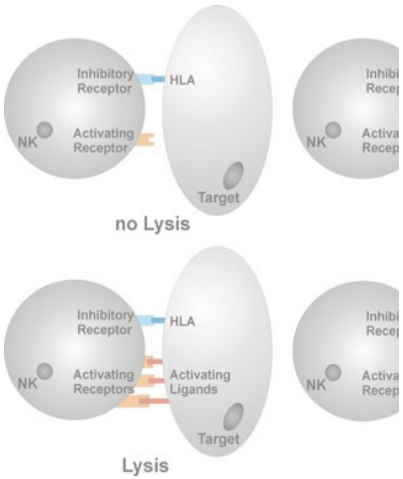


*HG. Ljunggren and K. Karre, Immunol Today **11**, 237 (1990)*

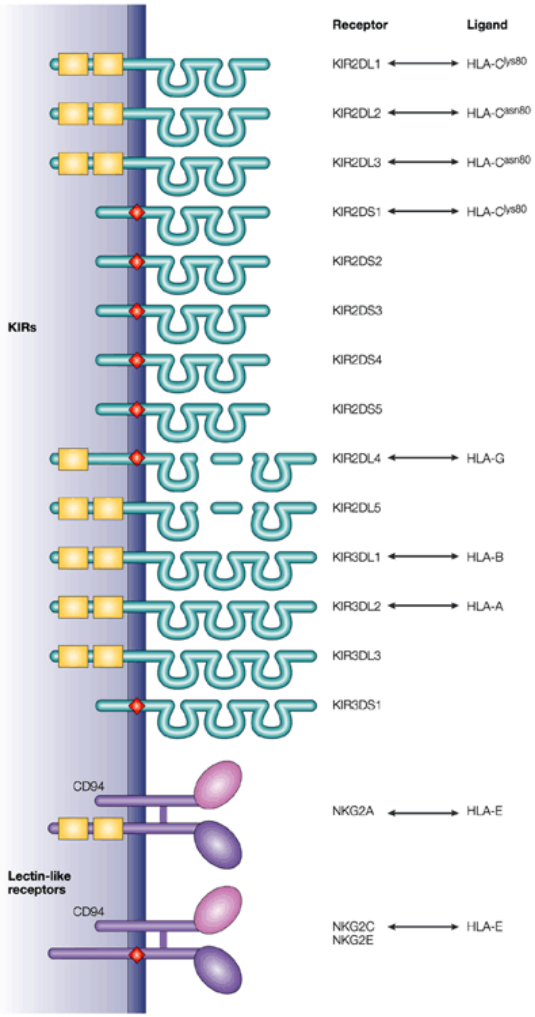
NK ligands


Inhibitory Receptor
HLA
NK
Activating Receptor
Target
no Lysis
Inhibitory Receptor
NK
Activating Receptor
Activating Ligand
Target
Lysis
Inhibitory Receptor
HLA
NK
Activating Receptors
Activating Ligands
Target
Lysis
Inhibitory Receptors
HLA
NK
Activating Receptor
Activating Ligand
Target
no Lysis

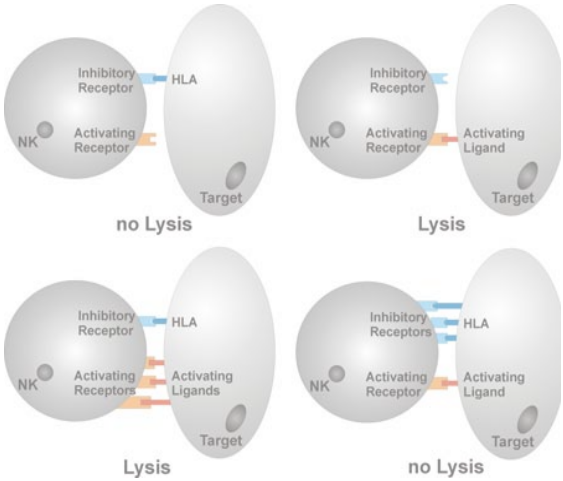
NK ligands



NK cell receptors



NK ligands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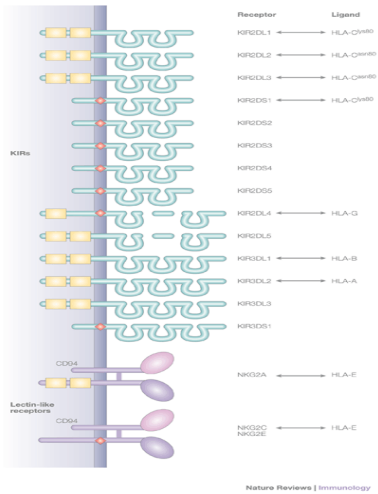
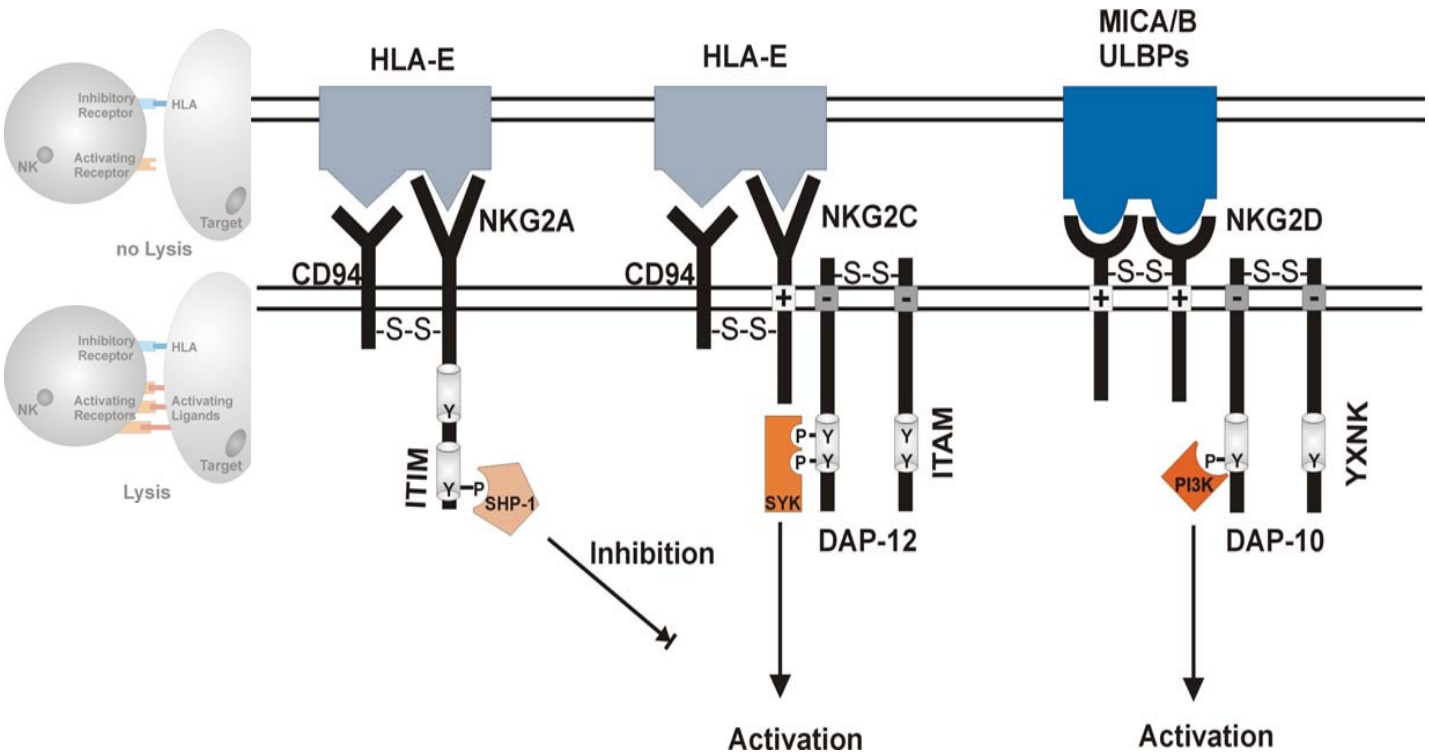

Receptor
Ligand
KIR2DL1
KIR2DL2
KIR2DL3
KIR2DS1
KIR2DS2
KIR2DS3
KIR2DS4
KIR2DS5
KIRs
KIR2DL4
HLA-G
KIR2DL5
KIR3DL1
HLA-B
KIR3DL2
HLA-A
KIR3DL3
KIR3DS1
CD94
NKG2A
HLA-E
Lectin-like receptors
CD94
NKG2C
NKG2E
HLA-E
Nature Reviews | Immunology

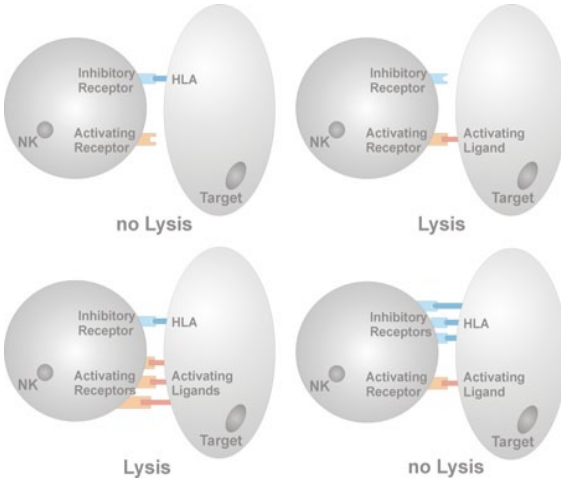
NK ligands

C-type lectin receptors



*V. Braud et al. Nature **391**:795 (1998); S. Bauer et al. Science **285**:727 (1999); J. Michaelsson et al. J Exp Med **196**:1403 (2002)*

NK ligands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Activating Ligands
Target
Lysis
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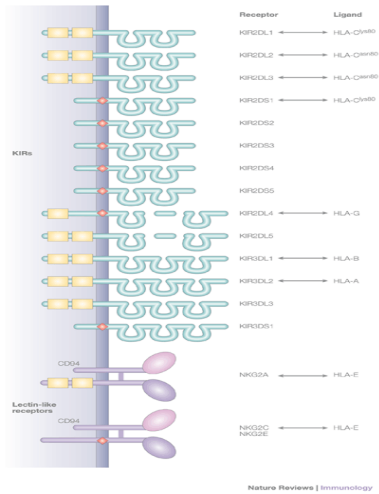
HLA
NK
Activating Receptor
Activating Ligand
Target
no Lysis


Receptor
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KIRs
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HLA-E
NKG2C
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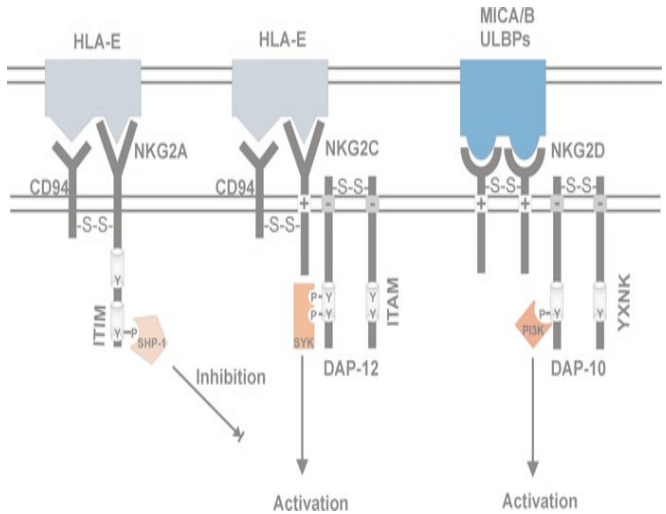
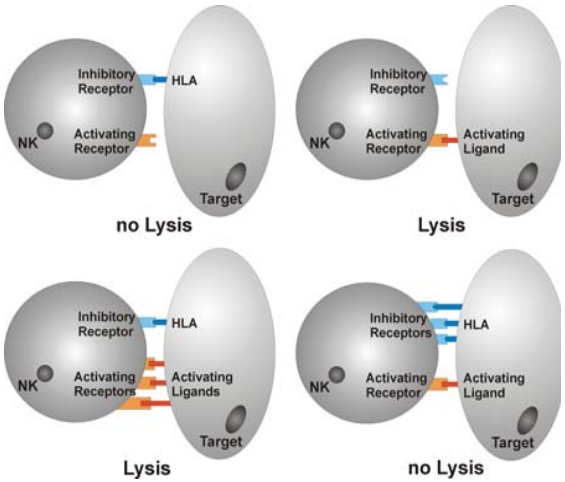
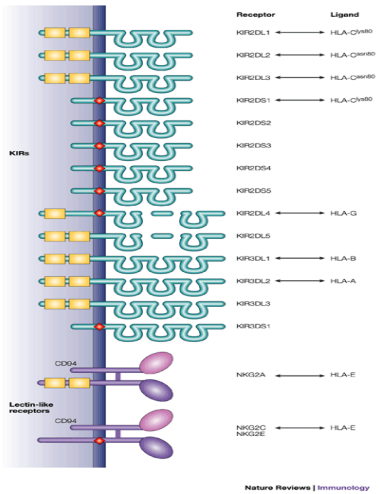
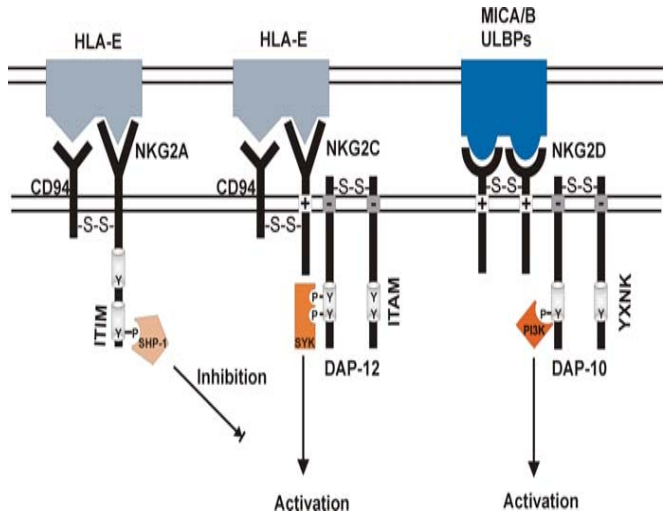

HLA-E
HLA-E
MICA/B
ULBPs
NKG2A
NKG2C
NKG2D
CD94
CD94
-S-S-
-S-S-
-S-S-
-S-S-
-S-S-
ITIM
SHP-1
SYK
ITAM
PI3K
YXNK
Inhibition
DAP-12
DAP-10
Activation
Activation

NK ligands





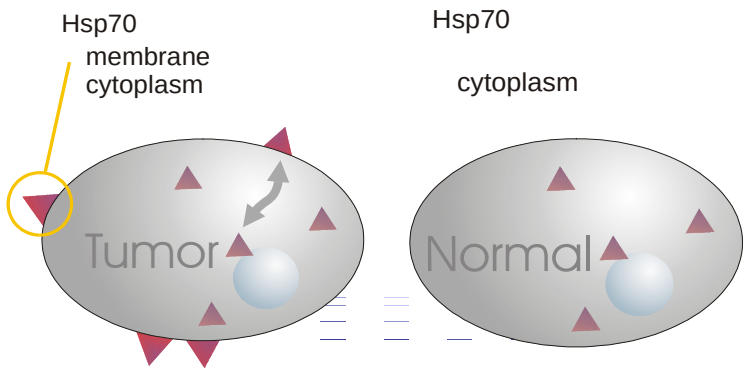


The NK cell response depends on the net effect of activating and inhibitory receptors with additional involvement of adaptor molecules

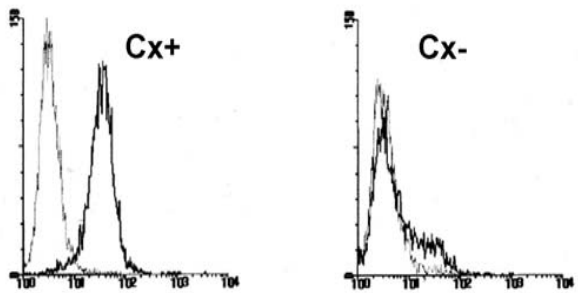
2. HSP70-NK interaction

Hsp70-NK interaction

Tumor-specifc HSP70 membrane expression



Hsp70 on tumor cells



Tissue bank

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

Hematological tumors

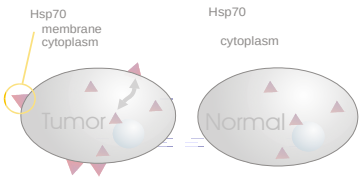
Metastases

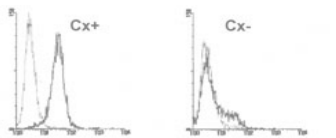
Methods

FACS
Surface biotinylation
Proteomic profiling

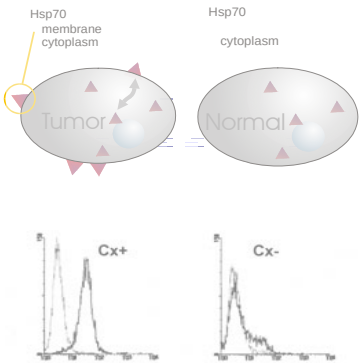
M. Ferrarini et al. (1992); Tamura et al. (1993); Chouchane et al. (1994); Multhoff et al. (1995), Altmeyer et al. (1996); Hantschel et al. (2000); Shin et al. JBC (2003)

Hsp70-NK interaction

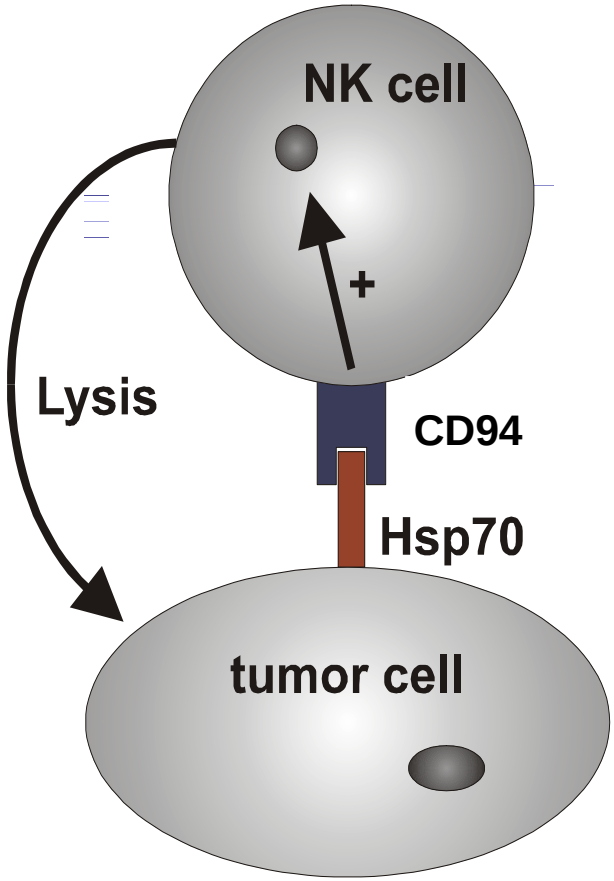




Hsp70-NK interaction



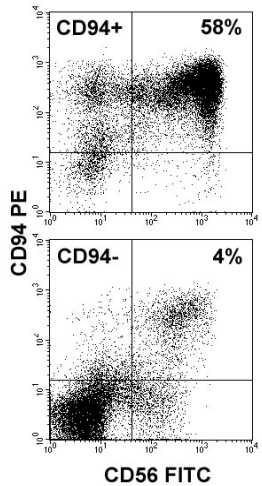
Receptor mediated HSP70-NK activation



NK cell proliferation ↑

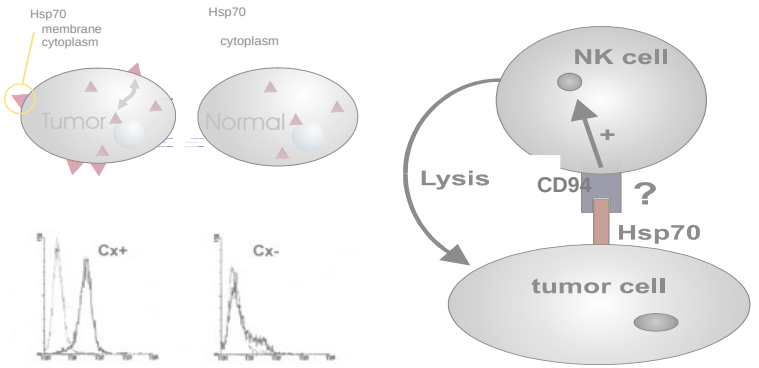
Cytolytic activity ↑

INF-γ production ↑



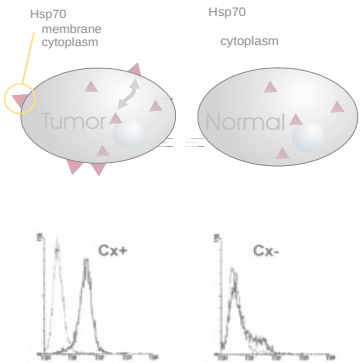
Multhoff et al. JI ***158:*** *4341 (1997); Multhoff et al. Exp Hematol* ***27****:1627 (1999); Gross et al. Cell Stress Chaperone* ***8****: 348, (2003);Gross et al. Biol Chem* ***384****: 267, (2003)*

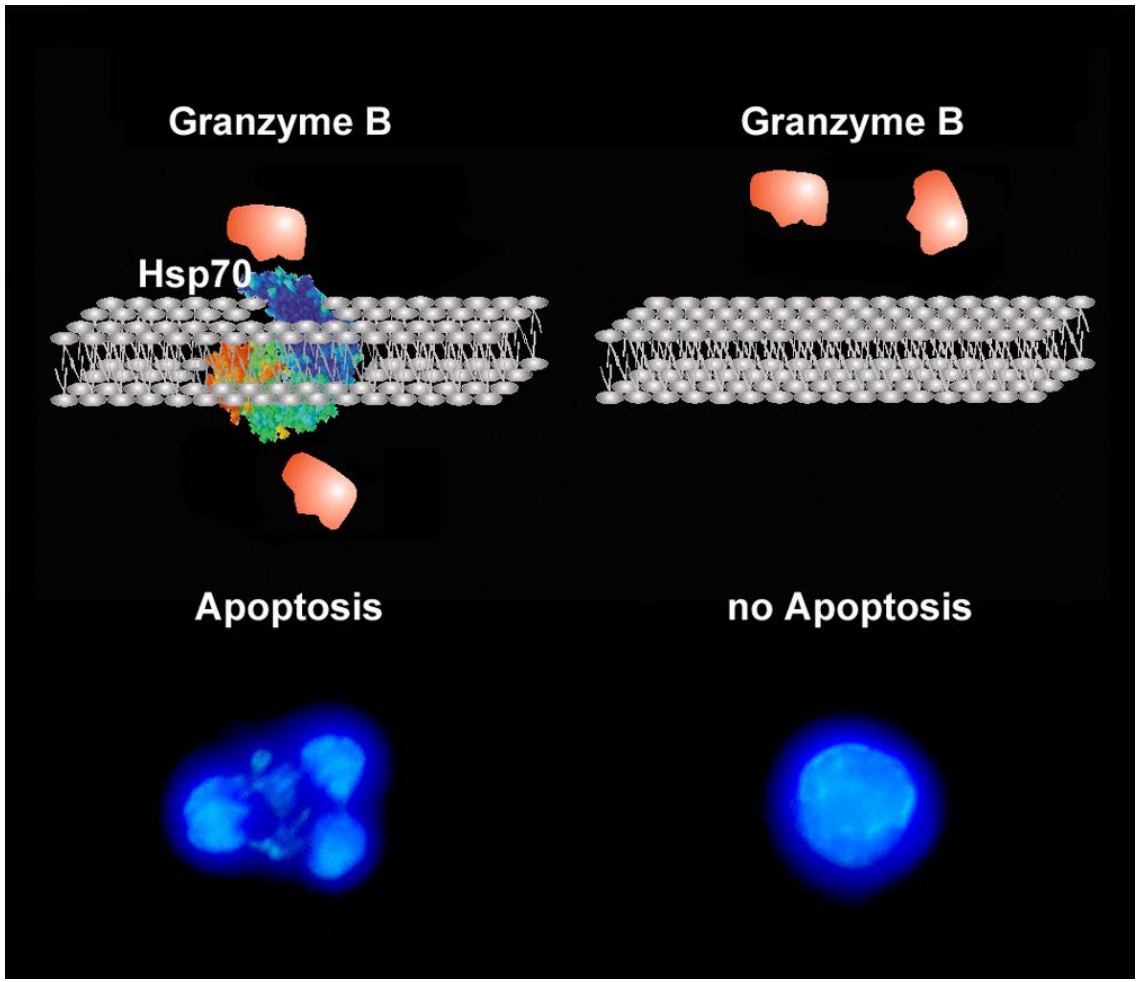
Hsp70-NK interaction



Hsp70-NK interaction

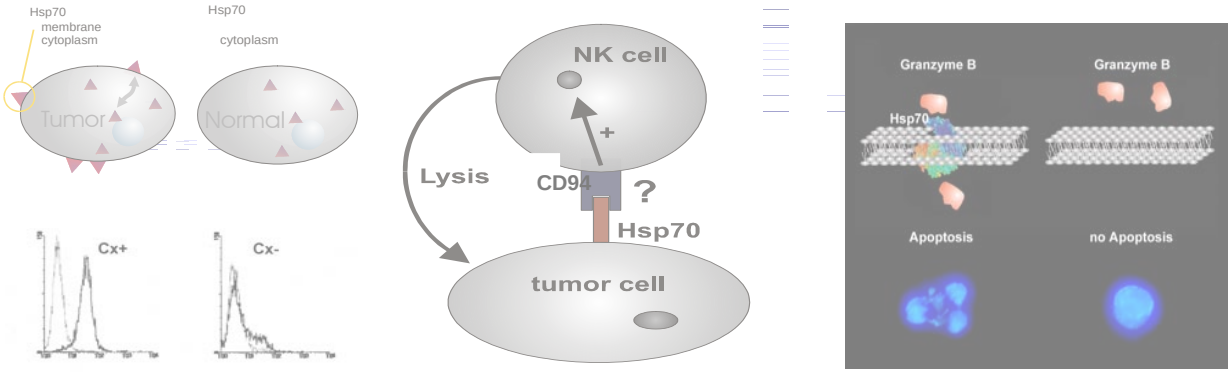
Mechanism of kill: Granzyme B (mi-APO) initiates apoptosis in Hsp70 positive tumors



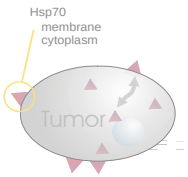


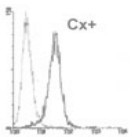
*Arispe et al. Cell Stress Chaperone (2002); Gross et al. J Biol Chem **17**: 41173, (2003)*

Hsp70-NK interaction



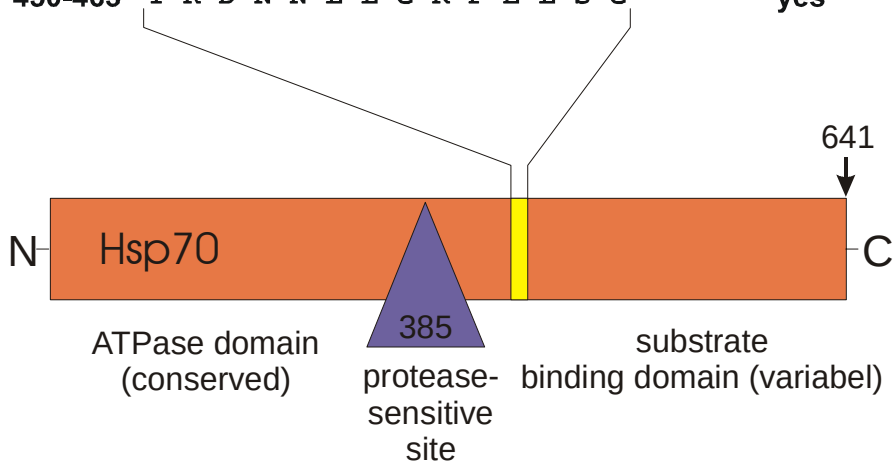
Hsp70-peptide TKD (aa 450-463) (ENKASTIM)





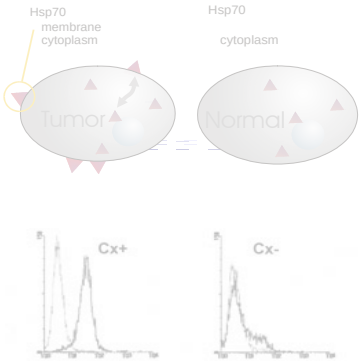
Epitope of the therapeutic Hsp70 specific antibody (mi-TUMEX)

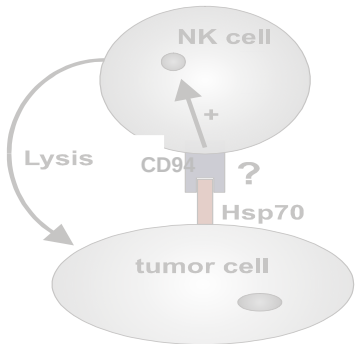
Name	aa	Sequence	NK Cell Stimulation
NLL	454-461	- - - - N L L G R F E L	no
GIPP	454-466	- - - - N L L G R F E L S G I P P	no
TKD	450-463	T K D N N L L G R F E L S G	yes

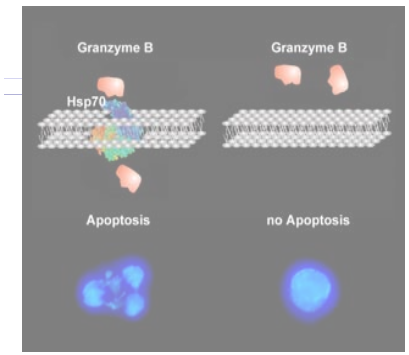


*Multhoff et al. Cell Stress Chaperone **6**: 337, (2001); Gastpar et al J Immunol **172**: 972 (2004)*

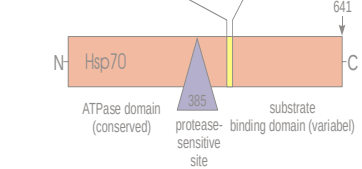
Hsp70-NK interaction



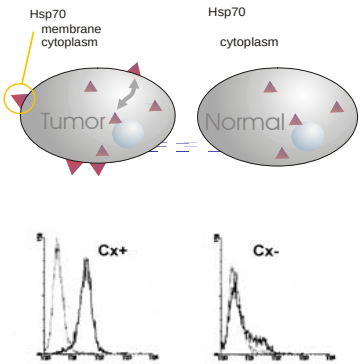


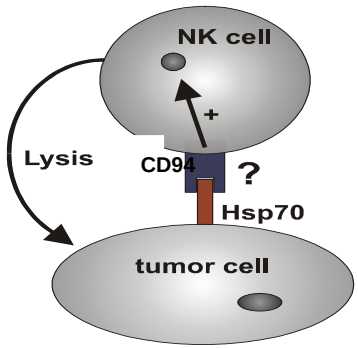


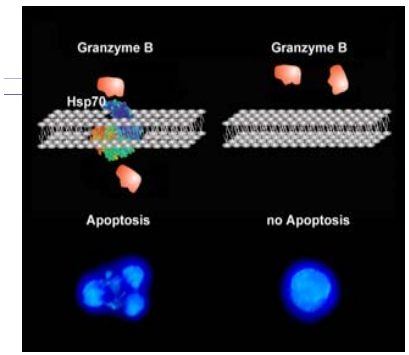
Name	aa	Sequence	NK Cell Stimulation
NLL	454-461	- - - - N L L G R F E L	no
GIPP	454-466	- - - - N L L G R F E L S G I P P	no
TKD	450-463	T K D N N L L G R F E L S G	yes

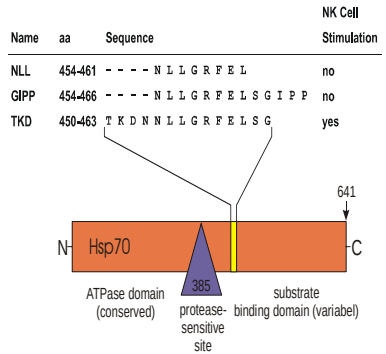


Hsp70-NK interaction







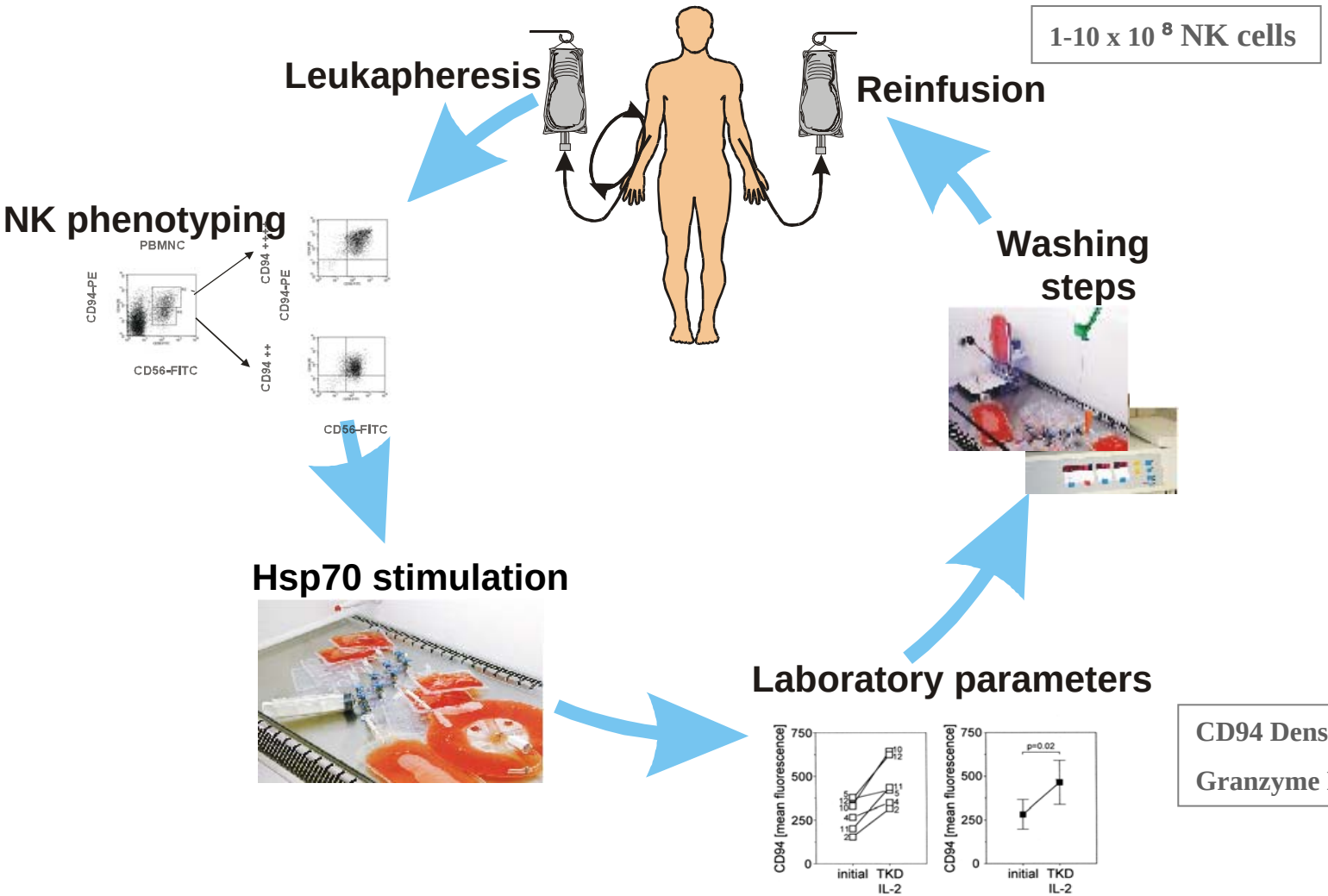


1995 **1997** **2001-2003** **2004-2005**

From the bench to the bedside

3. Clinical trials

Phase I clinical trial



*Krause et al. Clin Canc Res **10**: 3699 (2004)*

Phase I clinical trial

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

Patients:

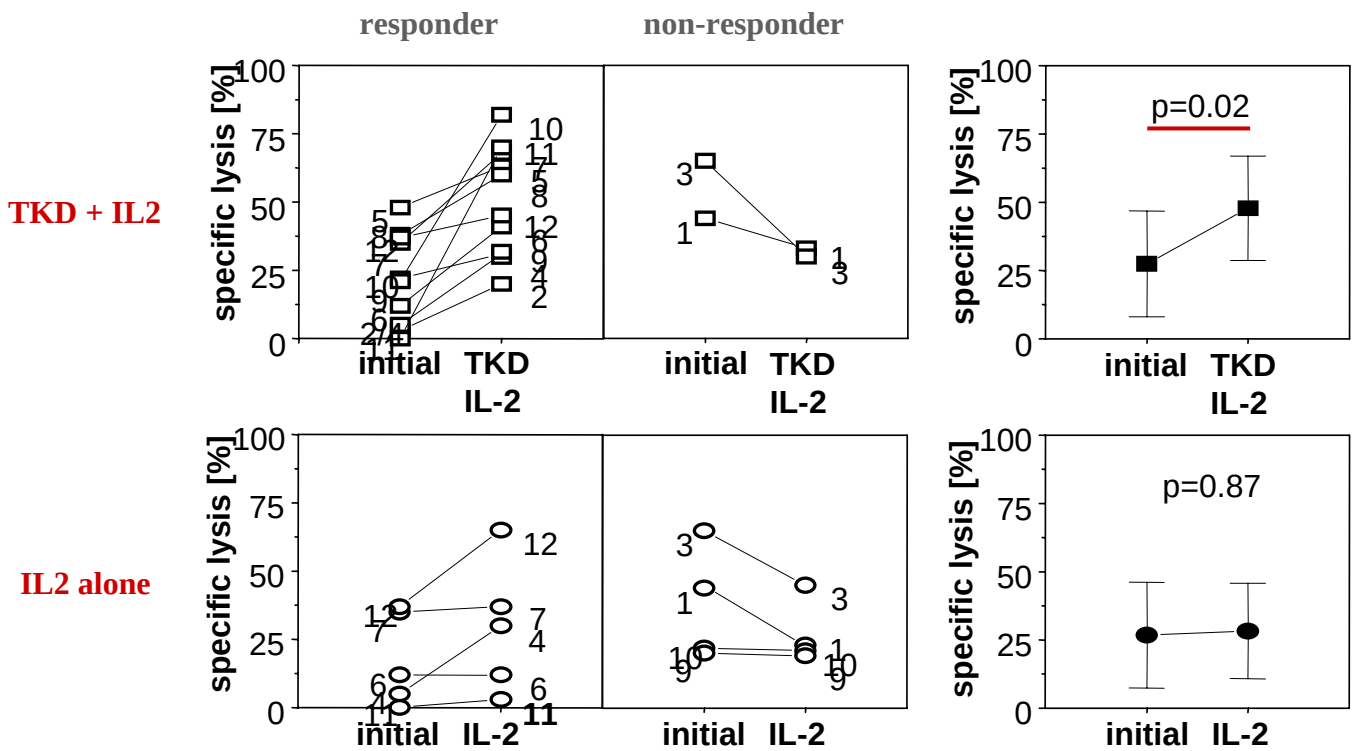
metastasised, therapy refractory colorectal cancer (n = 11) and non-small cell lung cancer (n = 1)

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 10^8, 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

*Krause et al. Clin Canc Res **10**: 3699 (2004)*

Phase I clinical trial

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



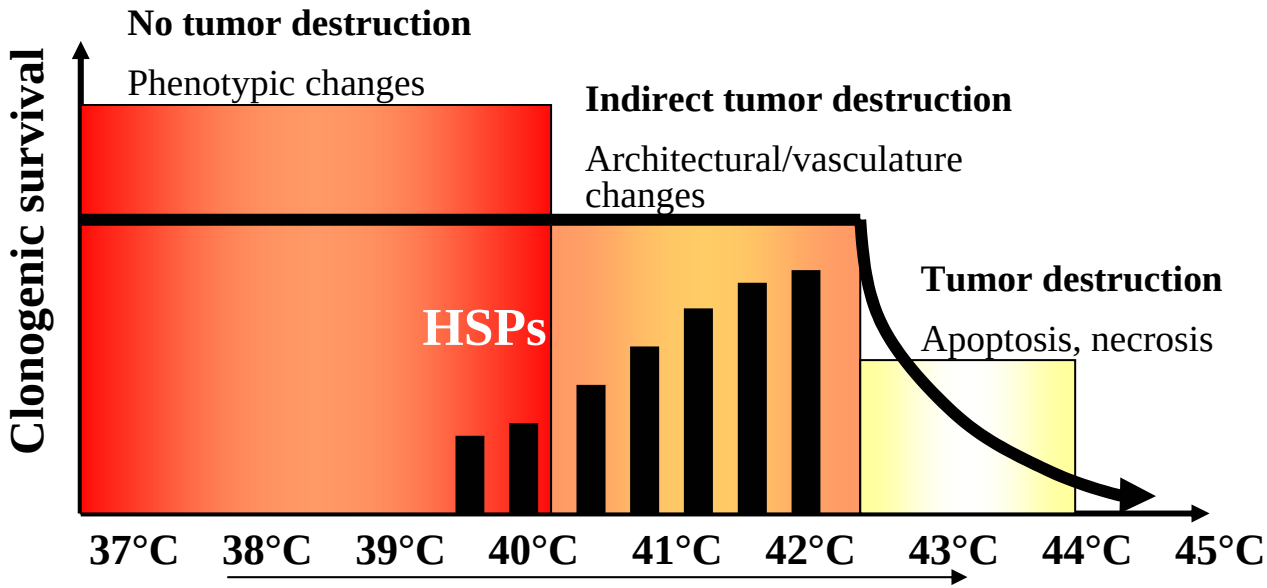
*Krause et al. Clin Canc Res **10**: 3699 (2004)*

Clinical studies on NK cell adoptive immunotherapy

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

4. Hyperthermia and NK transfer

Clinical hyperthermia

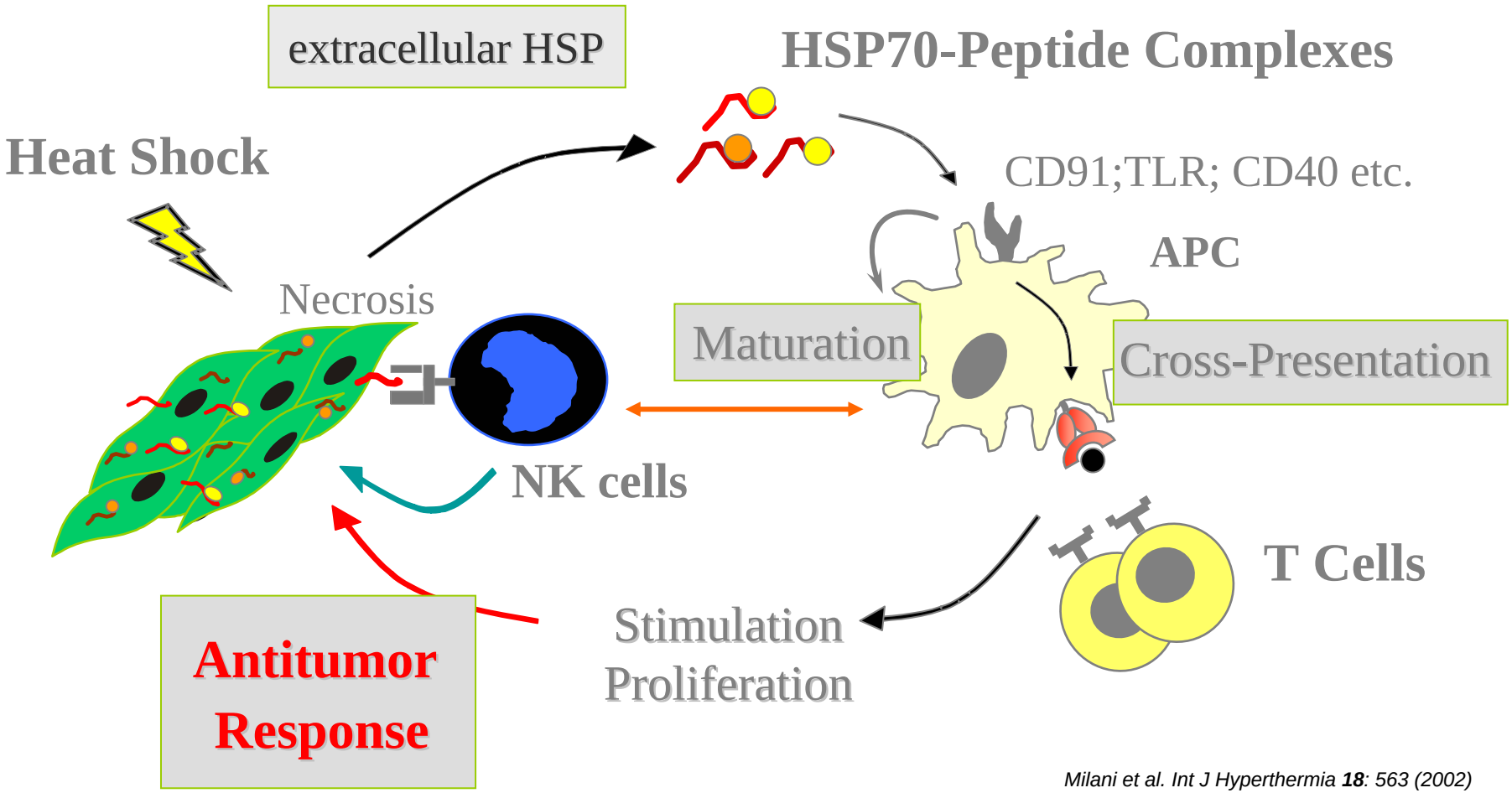


Immune response	Increased susceptibility to NK Maturation/Activation od DC	Increased lymphocytes migration/infiltration	Release of HSPs and HSP-PC
Molecular response	Cell cycle; DNA repair	Apoptosis	Necrosis
	Induction of intracellular heat shock proteins		
Clinical setting	Thermosensitisation of chemotherapy and radiation		
	Whole-body Hyperthermia		Regional hyperthermia

*Overgaard e al. Lancet 345:540 (1995); Van der Zee et al. Lancet **355**: 1119 (2000); Wendtner et al. J Clin Oncol. **20:**3156 (2202); Jones et al. J Clin Oncol **23**:7039 (2005)*

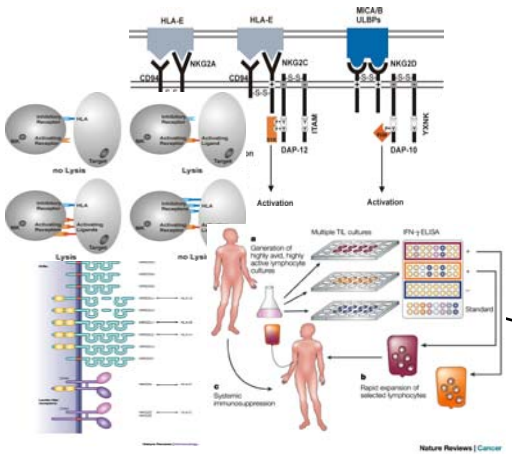
Hyperthermia: vaccination *in situ* ?

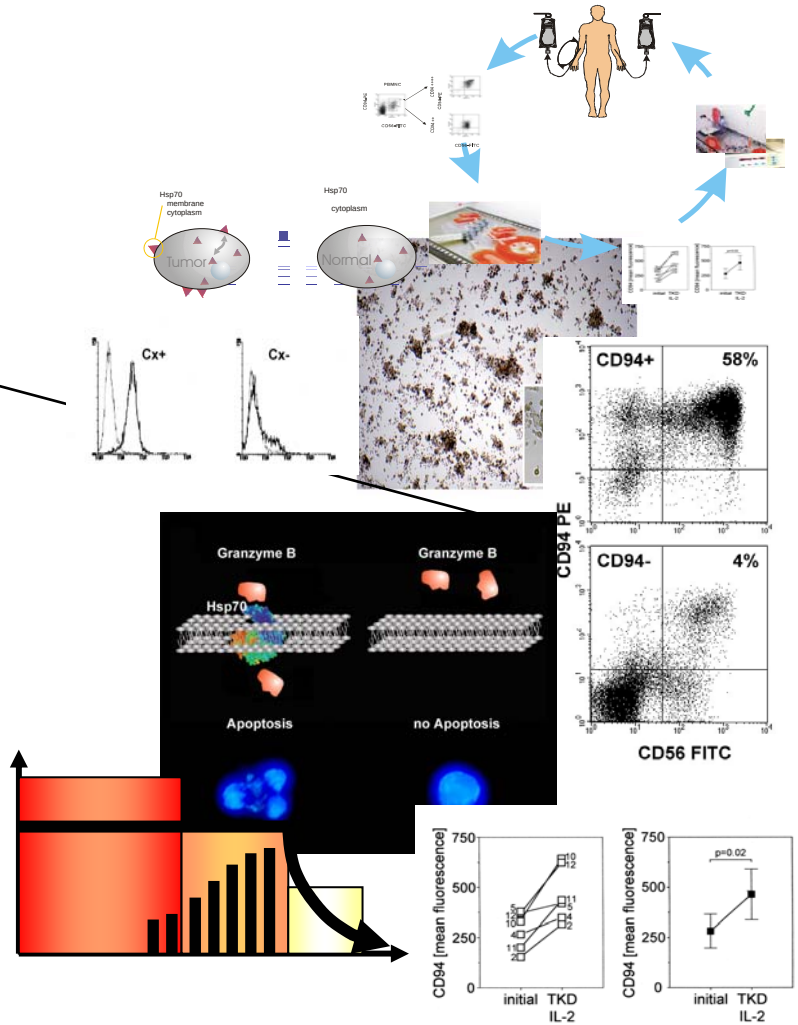
Tumor Necrosis and Release of HSP70 Activate the Immune System



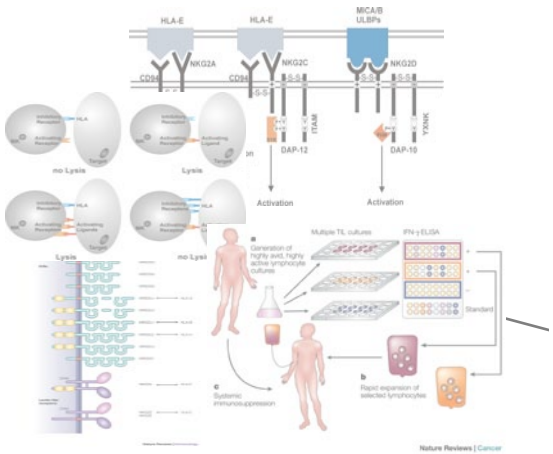
Milani et al. Int J Hyperthermia ***18****: 563 (2002)*

Can we specifically activate NK cells against tumor?

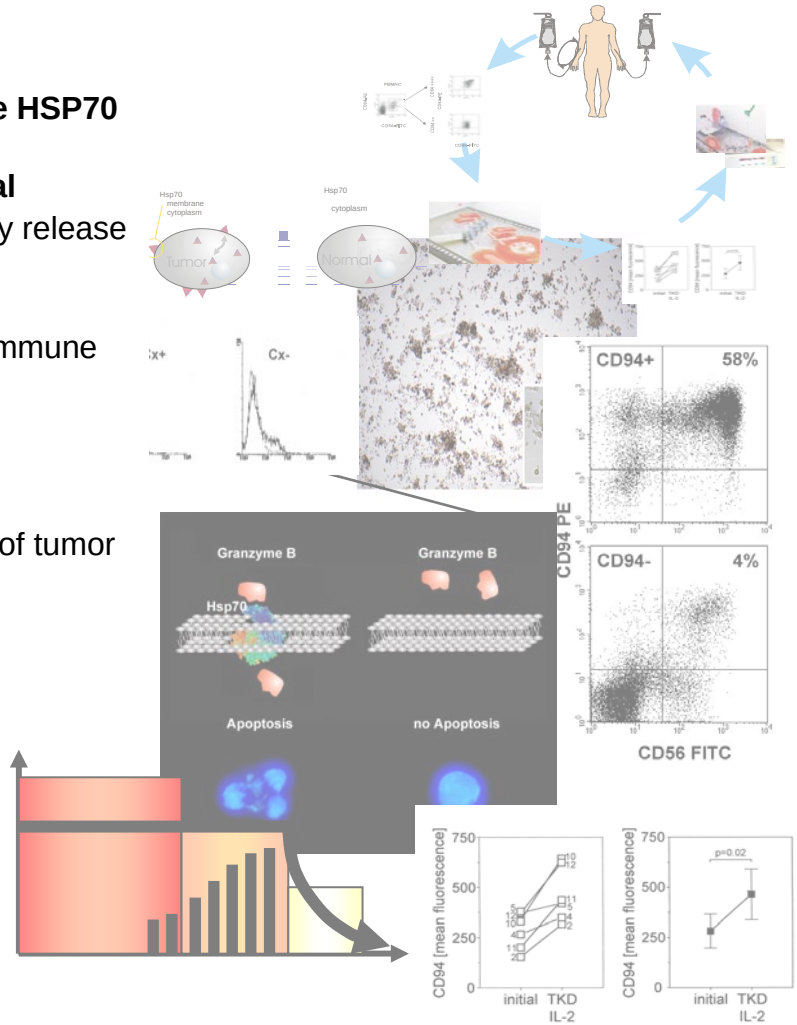




Can we specifically activate NK cells against tumor?



- 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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