The role of BioBran in NK-cell mediated treatment strategies in pediatric cancer

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Brussel
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Praha
Tschechische
Frankfurt am Main
Luxemburg
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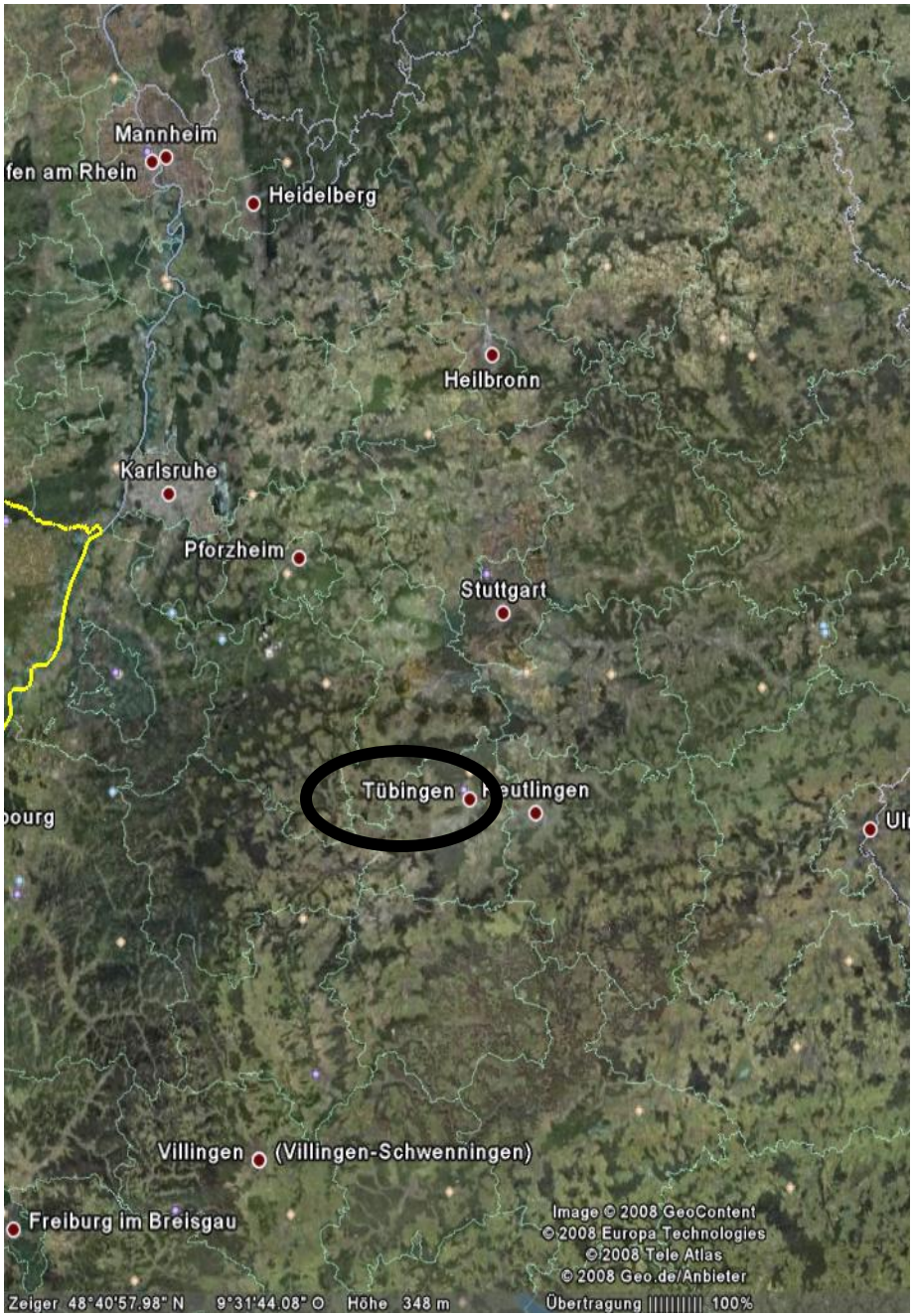
Mannheim
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City Hall

Major: B. Palmer
Green Party

Bursa (1480)

1514: P.Melanchthon, Reformer
1808: University Hospital

Stift (1260)

J. Kepler, Astronomer (1571-1630)
GWF Hegel, Philosopher (1770-1831

Catholic faculty

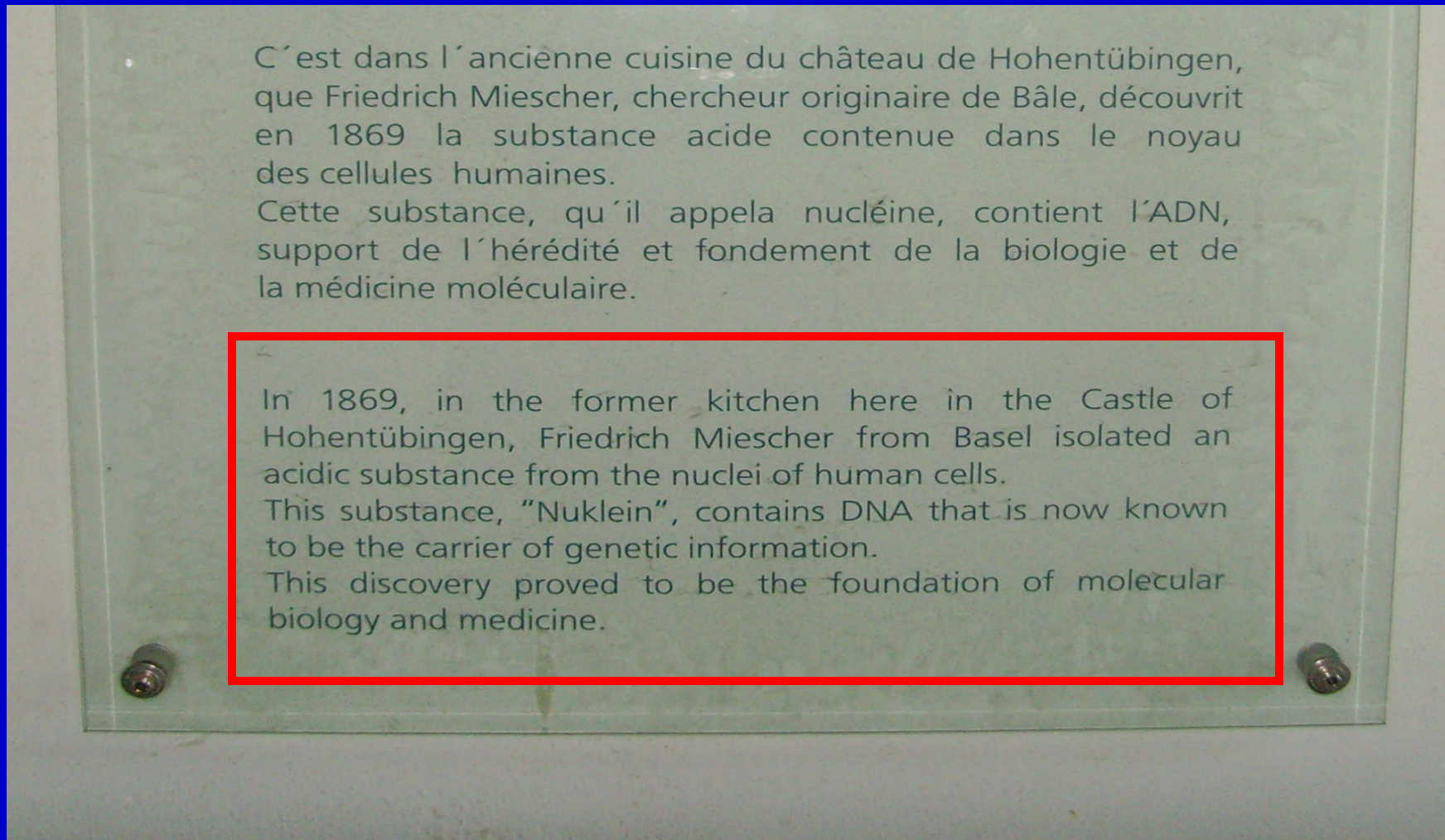
Pope Benedict (1966-69)
Prof. Hans Küng

Castle Hohentübingen

Graf Eberhard : Founder of the university in 1477

Castle kitchen

Isolation of an acidic substance from the nuclei of human cells by Friedrich Miescher in 1869 (Nuklein = DNA)



Erwin Bälz, MD (1849-1913)

1876-1905: Reformer of japanese medicine

Official physician to emperor Meji

Erwin Bälz,
you have awakened japanese medicine
to its first blossing.

(internat. Medical Society Japan)

The father of immunotherapy of cancer

William B. Coley (1862 – 1936)

Figure 1. William B. Coley (1862-1936) from *Trans Am Surg Assoc* 54(1936):415. Courtesy of the Welch Library of the History of Medicine.

Coley's Toxins (also called Coley's toxin,[1] Coley's vaccine,[2] Coley vaccine or Mixed Bacterial Vaccine) is a mixture consisting of killed bacteria of species ***Streptococcus pyogenes*** and *Serratia marcescens*, named after William Coley, a surgical oncologist who developed the mixture in the late 19th century as a treatment for cancer.

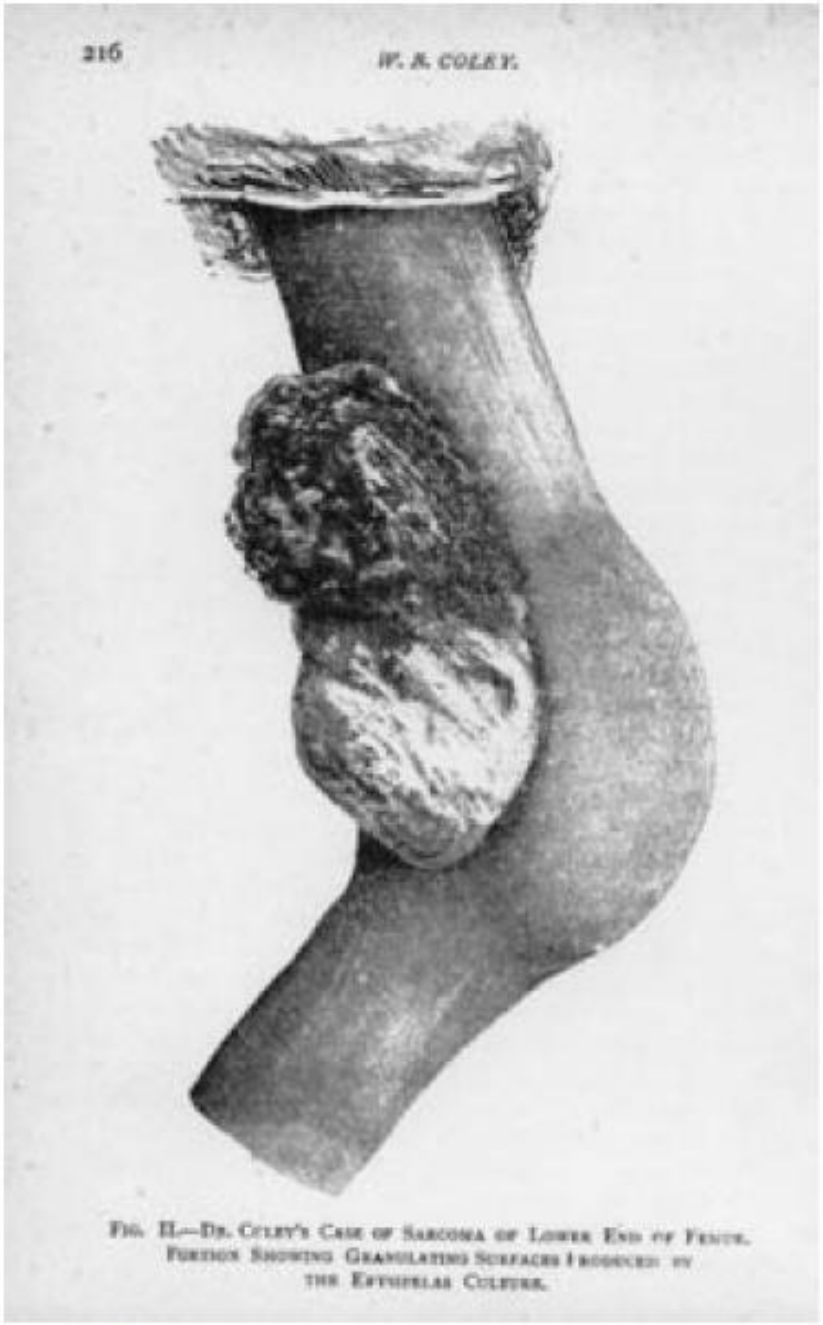
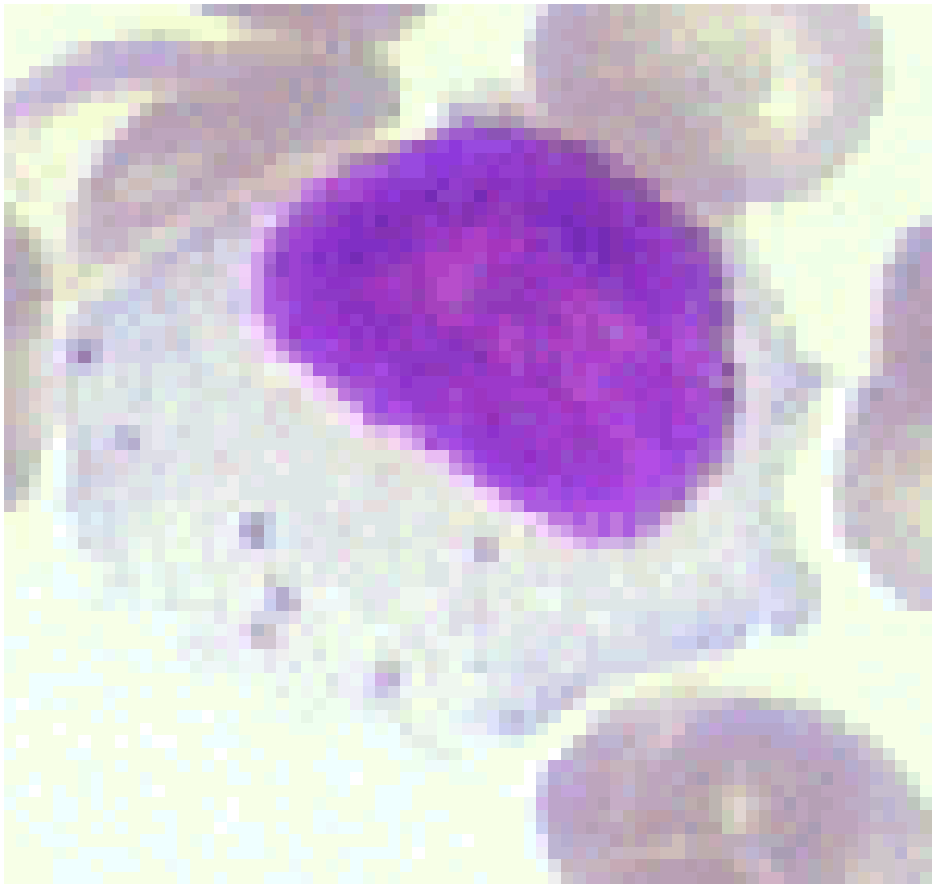


Figure 2. Drawing of Coley's first bone sarcoma case treated with his toxins. Courtesy of *Annals of Surgery*/Lippincott.

Immune systen

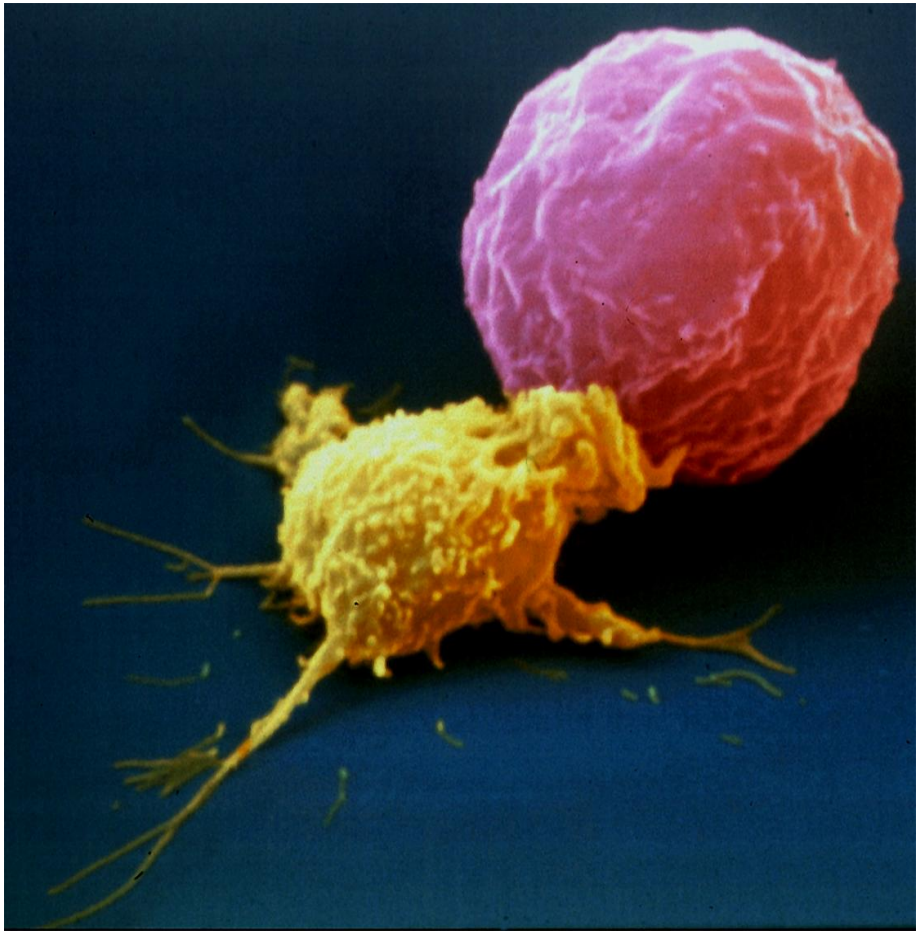
Innate immune system	Adaptive immune system
Natural Killer (NK)cells	T-lymphocyte system

Natural Killer (NK) cells



NK cell attacks a leukemic blast (K562 Erythroleukemia)

NK Assay



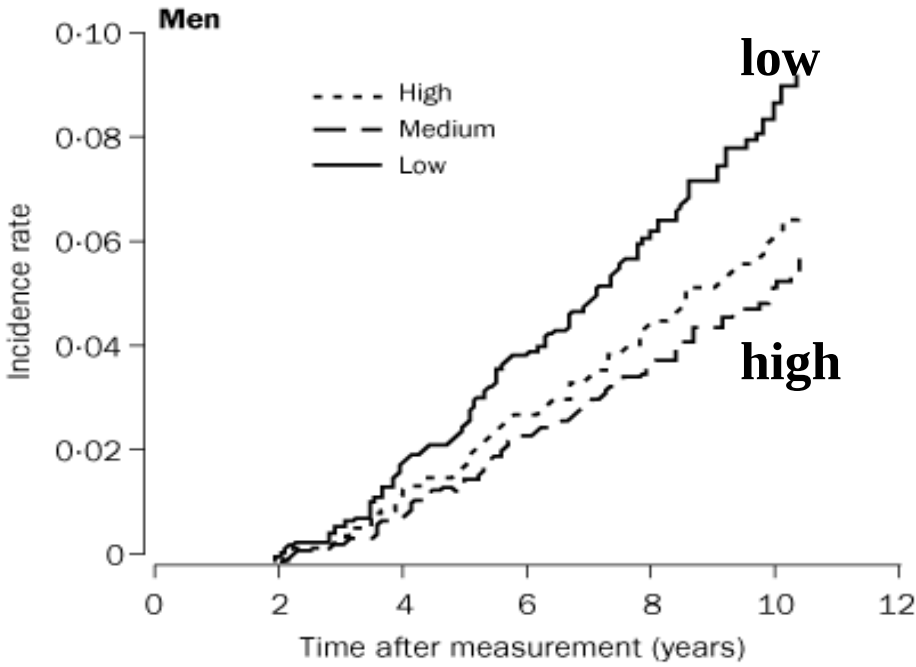
Eyeofscience, Reutlingen

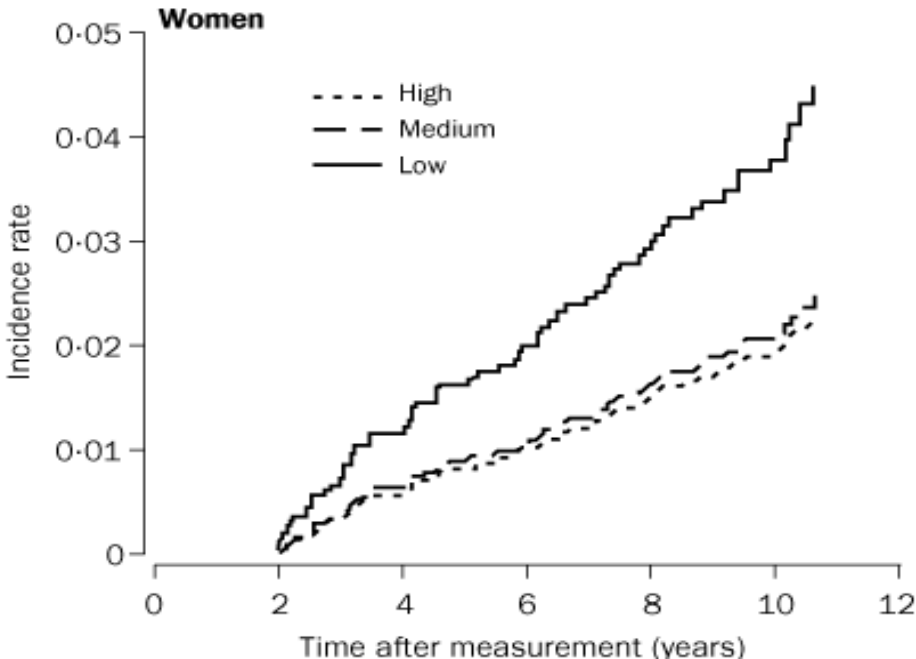
Natural Killer (NK) cells lyse cells that are deficient in expression of class I MHC proteins

Anti-tumor effect of NK cells in population study

- 3625 Japanese residents
- 1986 - 1990
- NK against K562,
- Prospective follow-up for a median of 11 year for incidence of cancer

Lancet 356:1795; 2000





How do NK cells recognize tumor cells?

How to recognize a foreign submarine
K.Kärre, Immunological Reviews 1997; 155:5-9

Sweden solves Cold War ‘submarine’ mystery

In 1982, Sweden claimed that it had recordings from a Soviet submarine in the waters of the Stockholm archipelago. The recording was made during a submarine hunt by the Swedish military on October 12th, 1982. It strengthened suspicions that Soviet subs were intruding in Swedish waters.

Which is the best surveillance system for submarines?

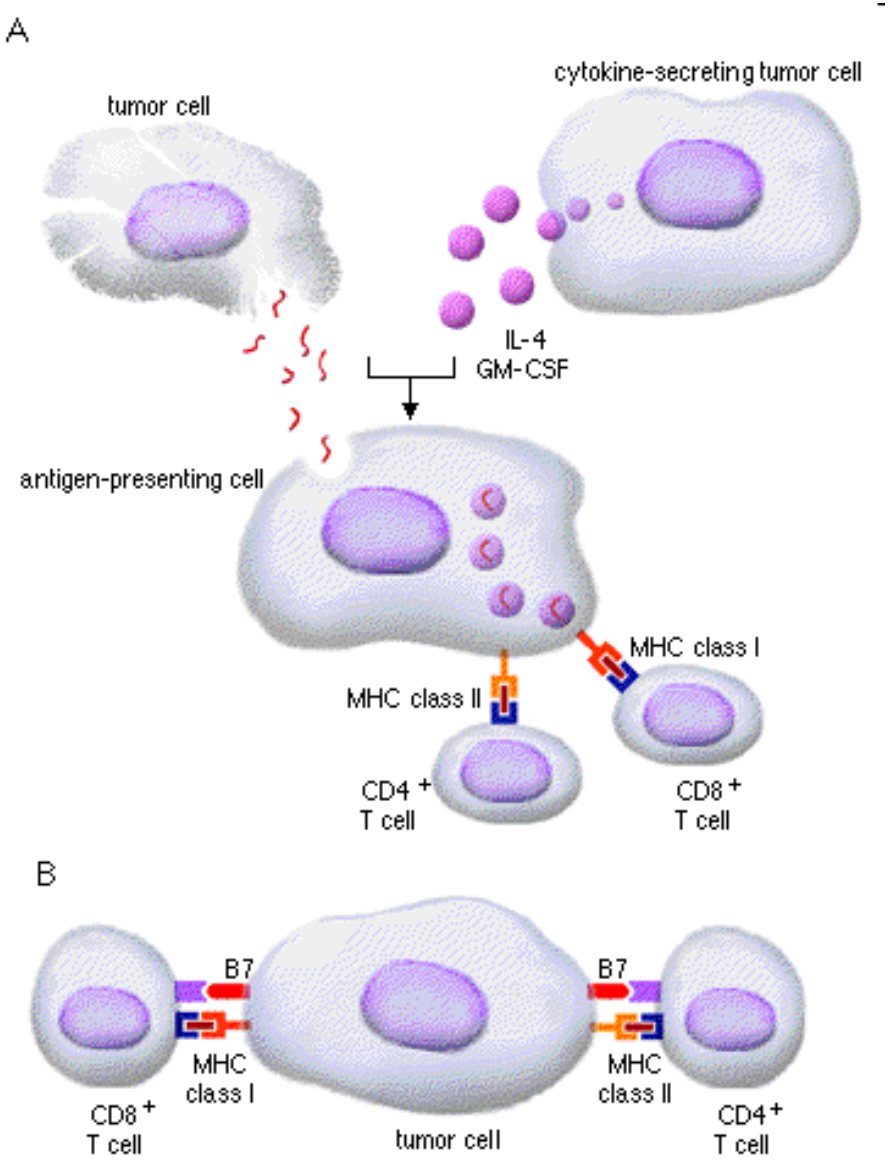
- Local fishers get a thick book with thousand of pages with the pictures of all submarine of the world.
- When you see a submarine thats in the book:

Call the marine

- Local fishers get one page with the pictures of the three swedish submarines.
- When you see something strange in the water and it looks like one of the three objects:

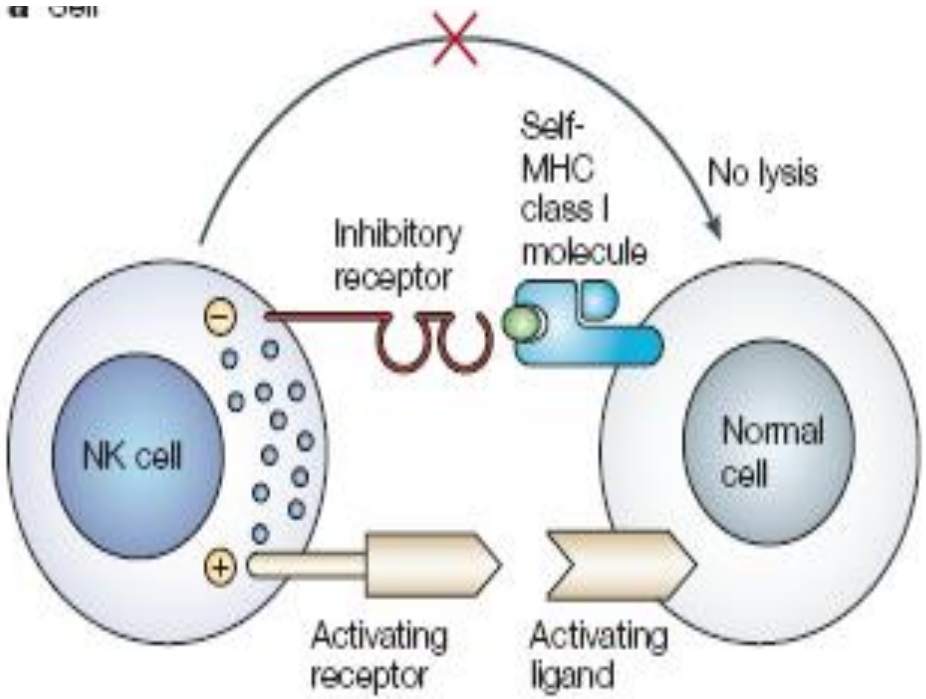
Do no call the marine

Call the marine

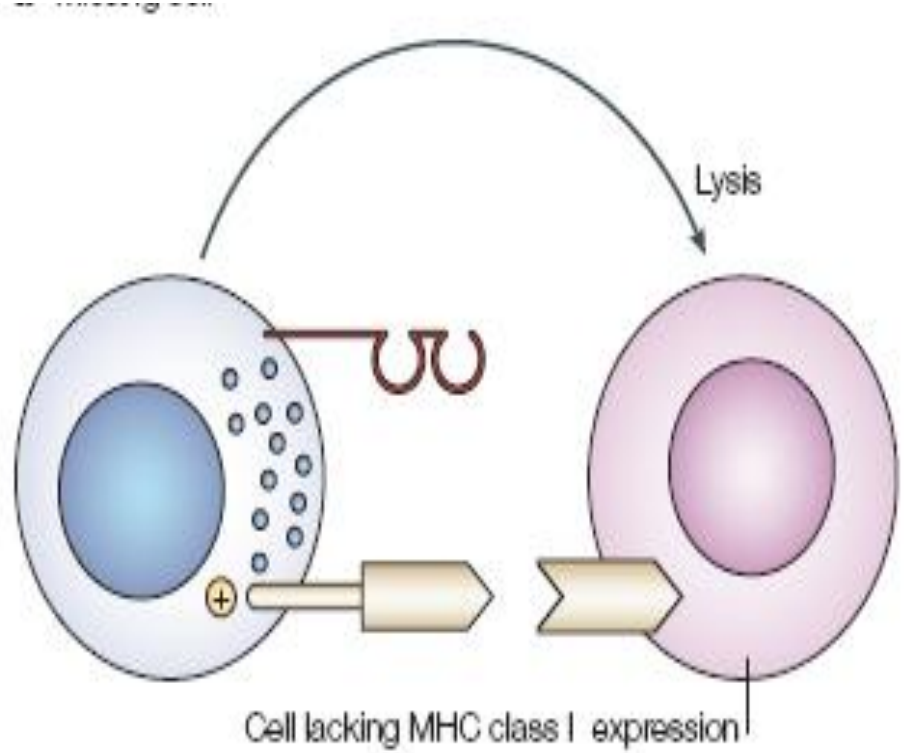


Nk cells have inhibitory receptors which recognize HLA class I antigens

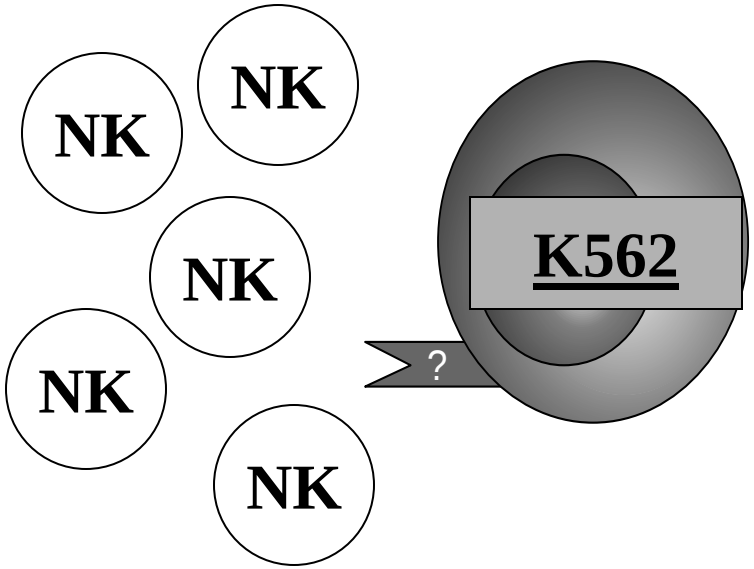
Do not call the marine



Lack of HLA class I:
Nk cells recognize the cell as foreign (missing self)



Why are K 562 good target cells?

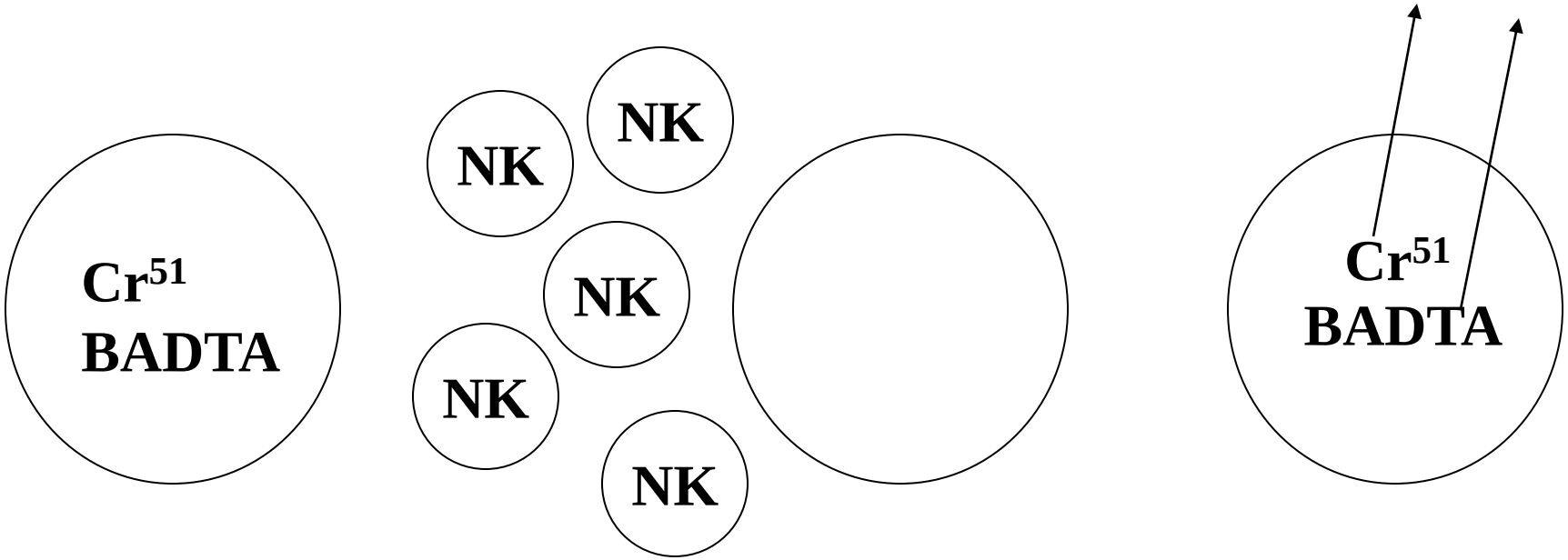


K562 (Erythroleukämie):

They lack HLA classI

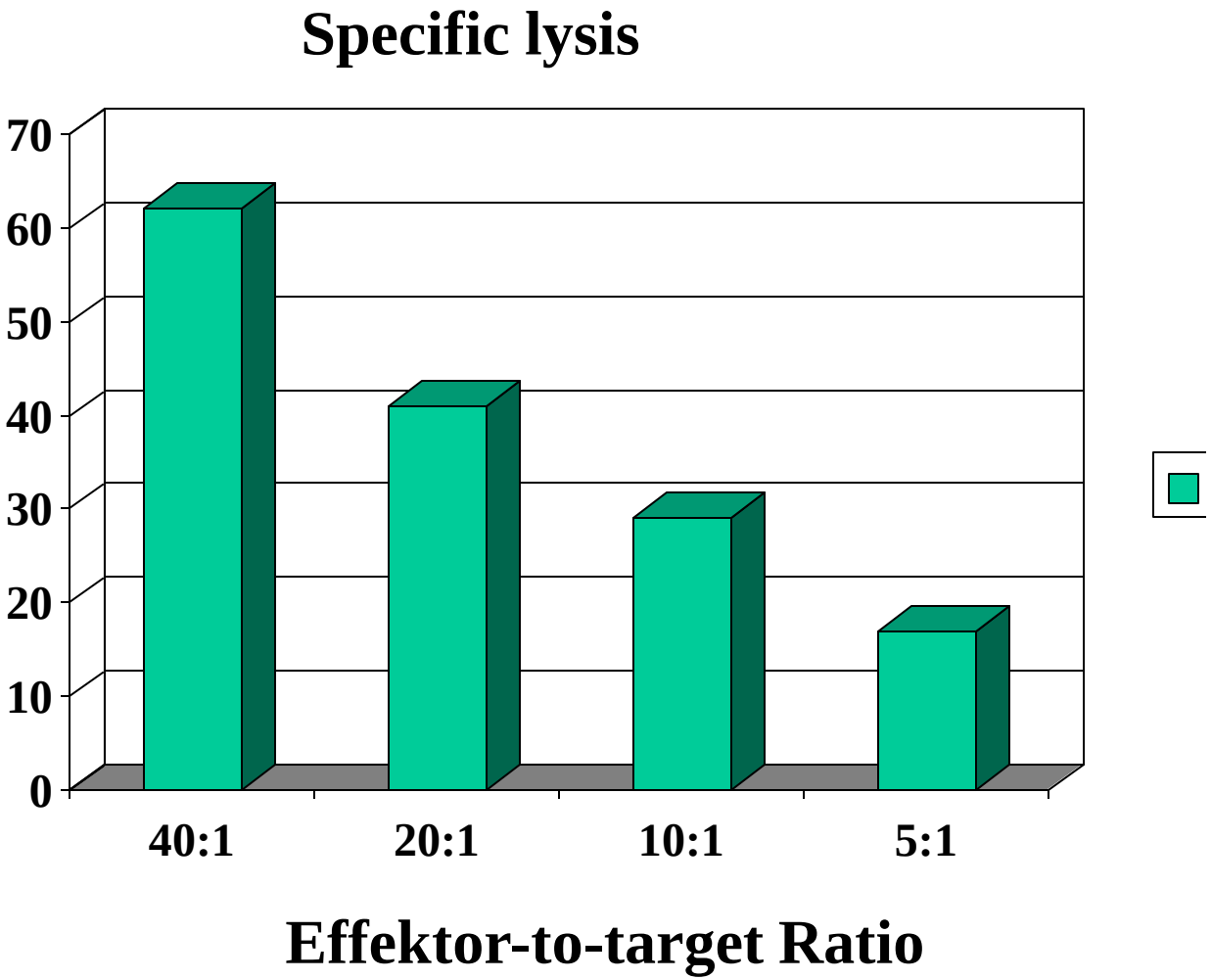
How to measure NK activity?

K562 Erythroleukämie

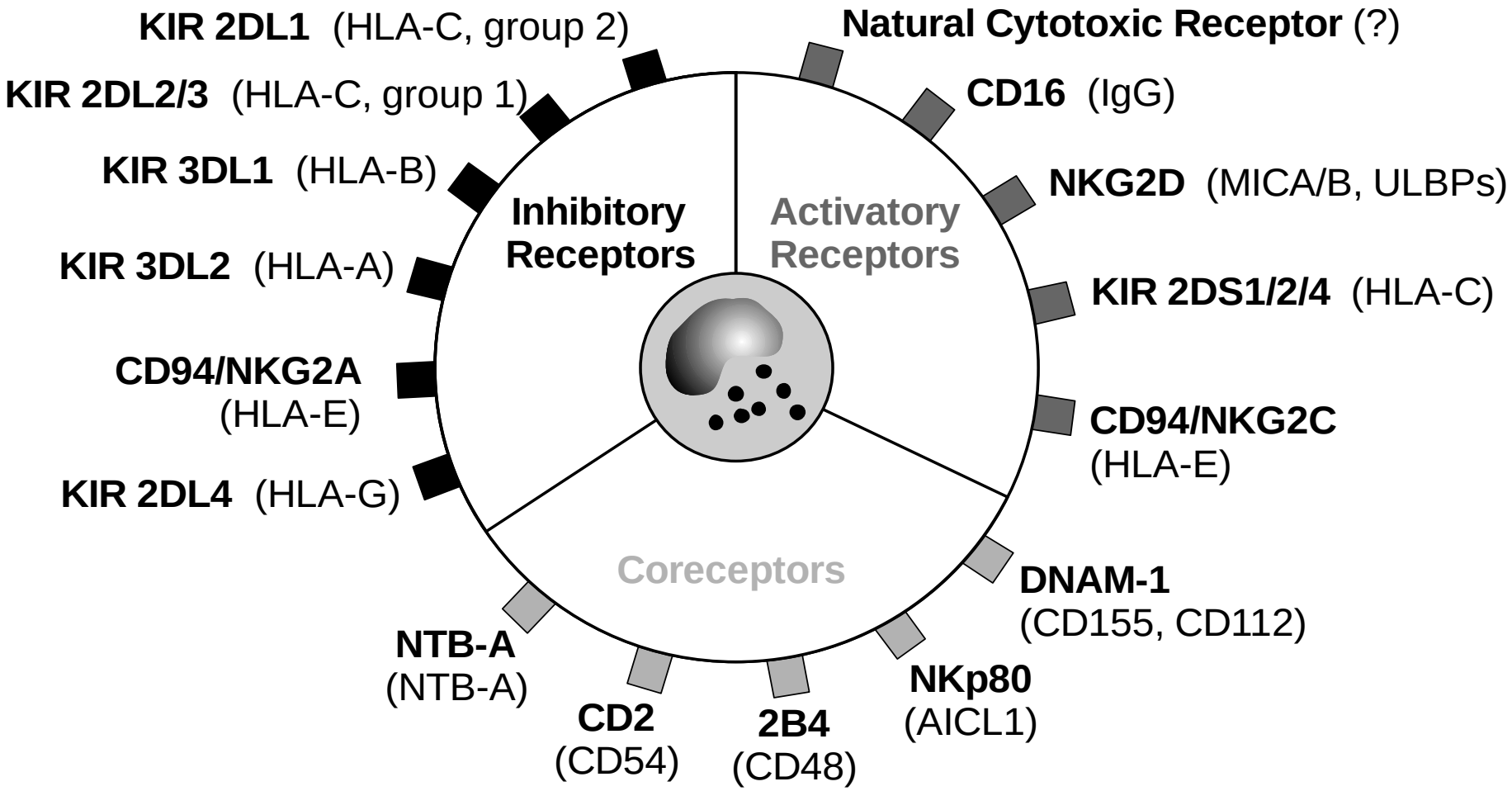


Effektor-to-target ratio

Cytotoxicity

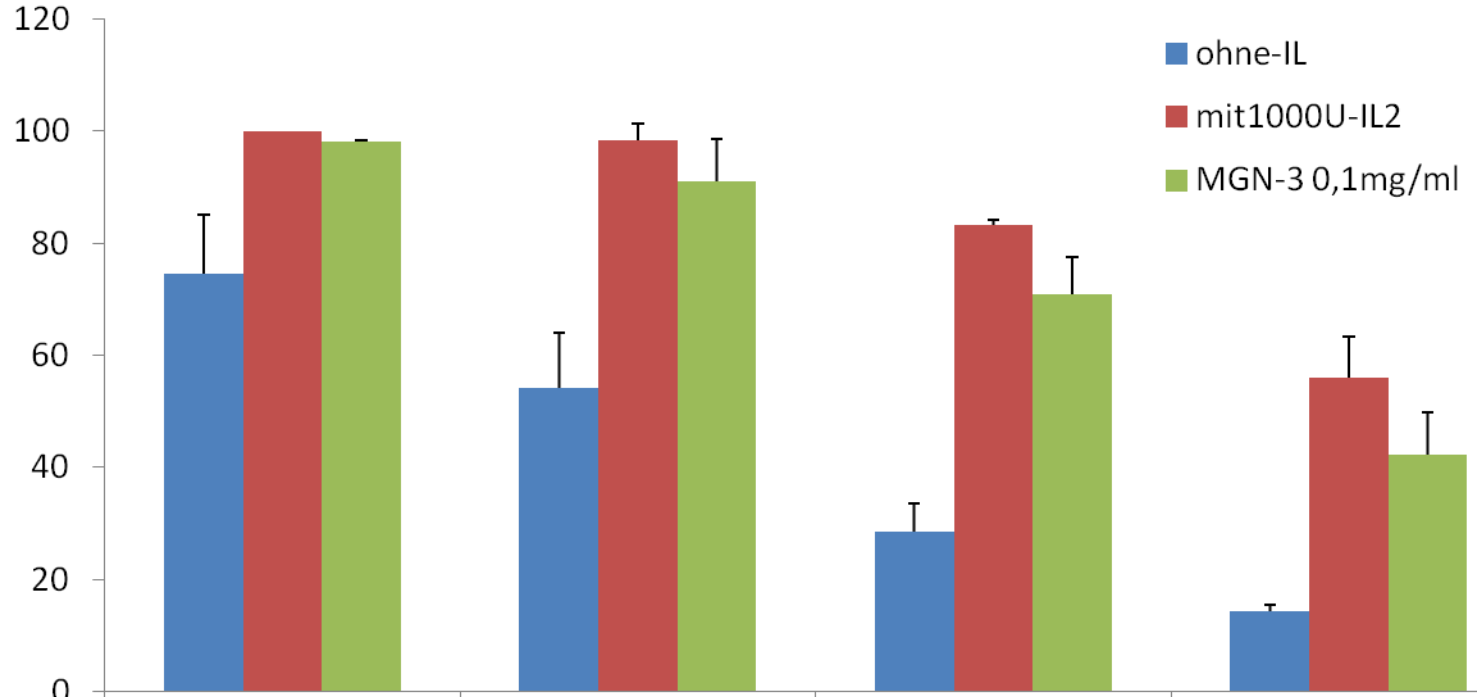


Inhibitory, activatory and Coreceptors on NK cells



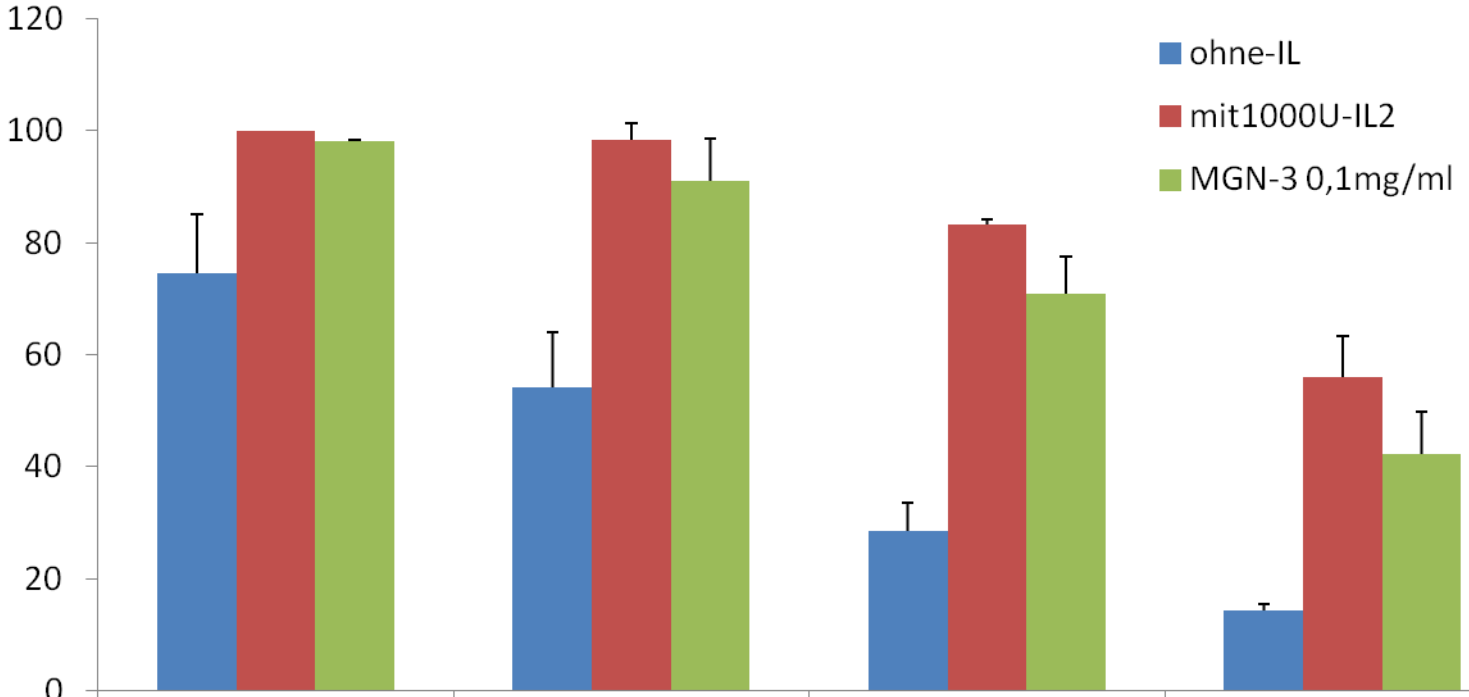
BioBran activates NK cells in vitro

NK Activity of healthy donors against K 562



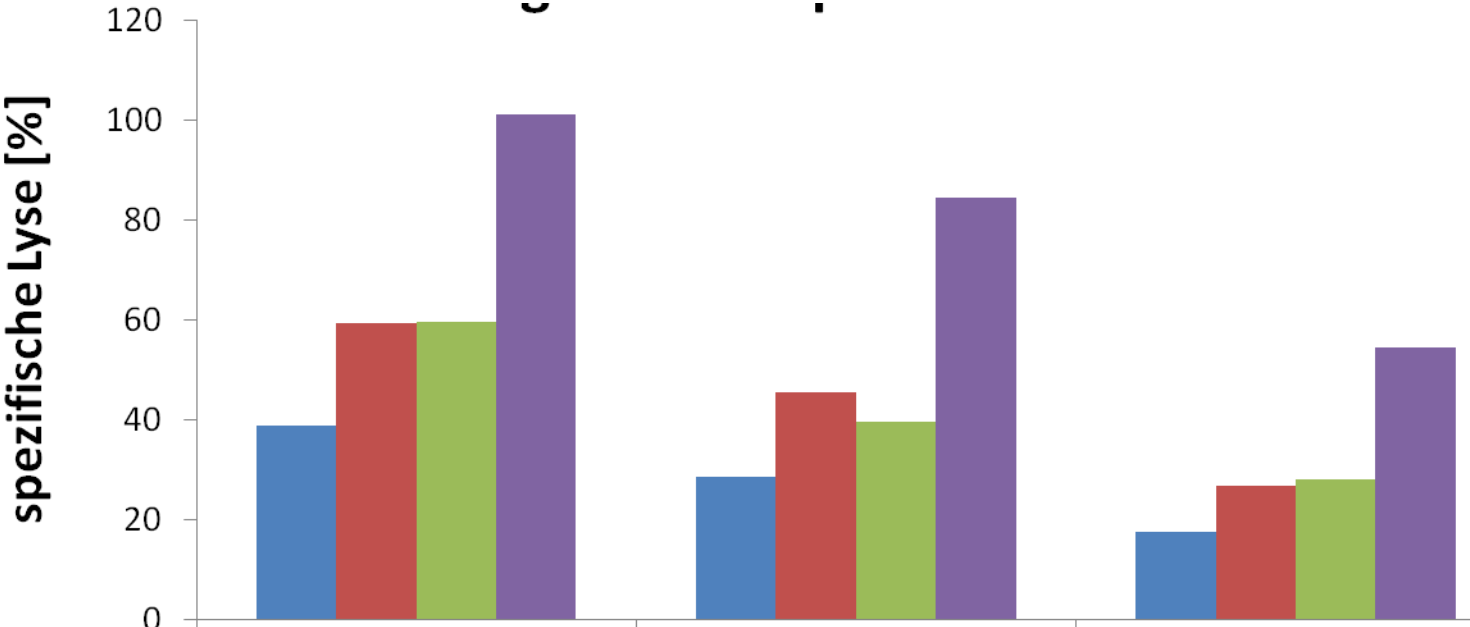
	20:1	10:1	5:1	2,5:1
ohne-IL	75	54	28	14
mit1000U-IL2	100	98	83	56
MGN-3 0,1mg/ml	98	91	71	42

NK Activity of healthy donors against K 562



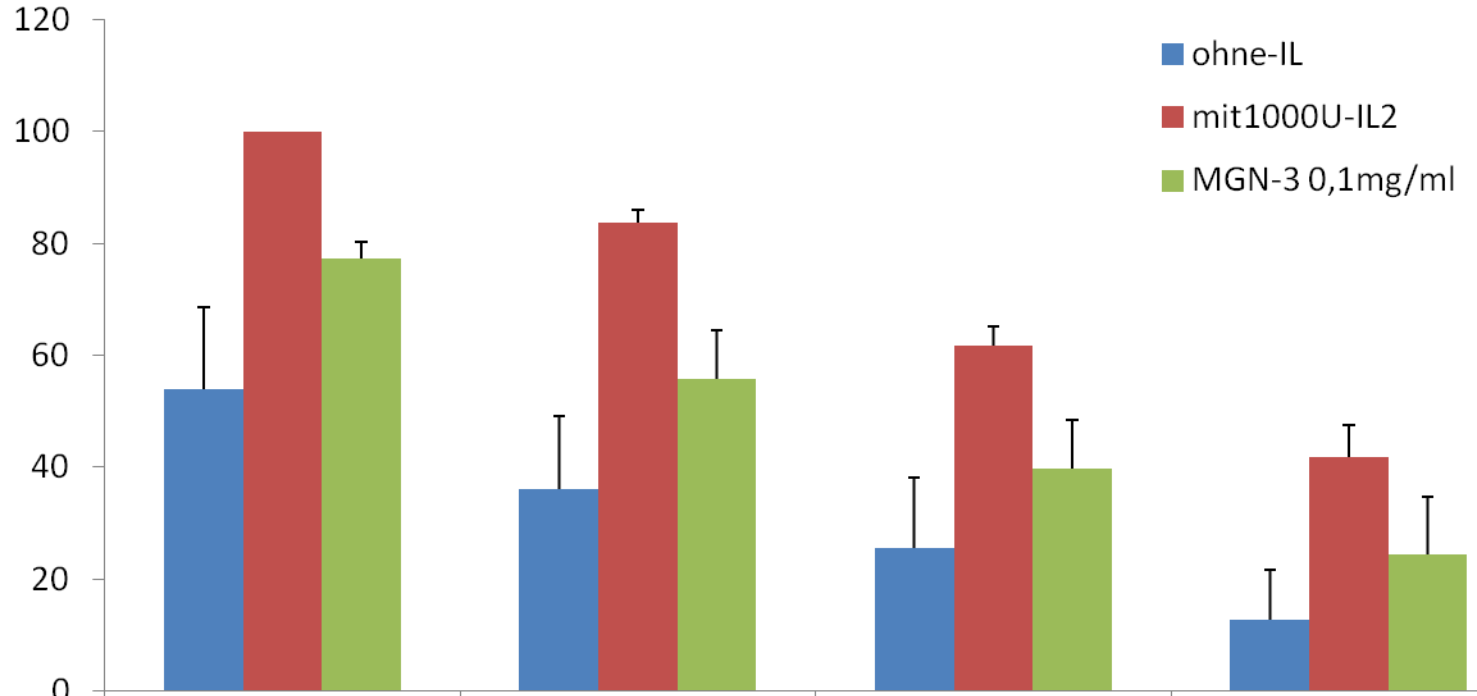
	20:1	10:1	5:1	2,5:1
ohne-IL	75	54	28	14
mit1000U-IL2	100	98	83	56
MGN-3 0,1mg/ml	98	91	71	42

NK Activity of healthy donors against K 562



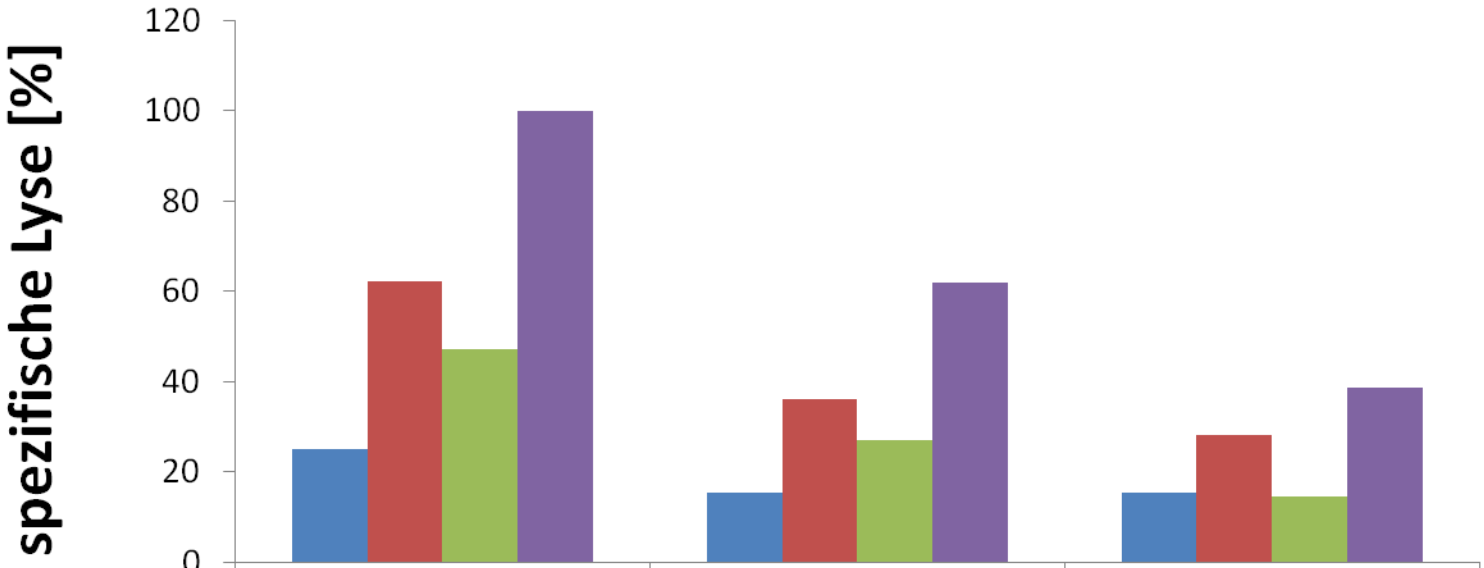
	20:1	10:1	5:1
nur NKC	39	29	17
40 U/ml IL2	59	46	27
0,1 mg/ml MGN3	60	39	28
40 U/ml IL2 + 0,1 mg/ml MGN3	101	85	54

NK Activity against Hepatoblastoma



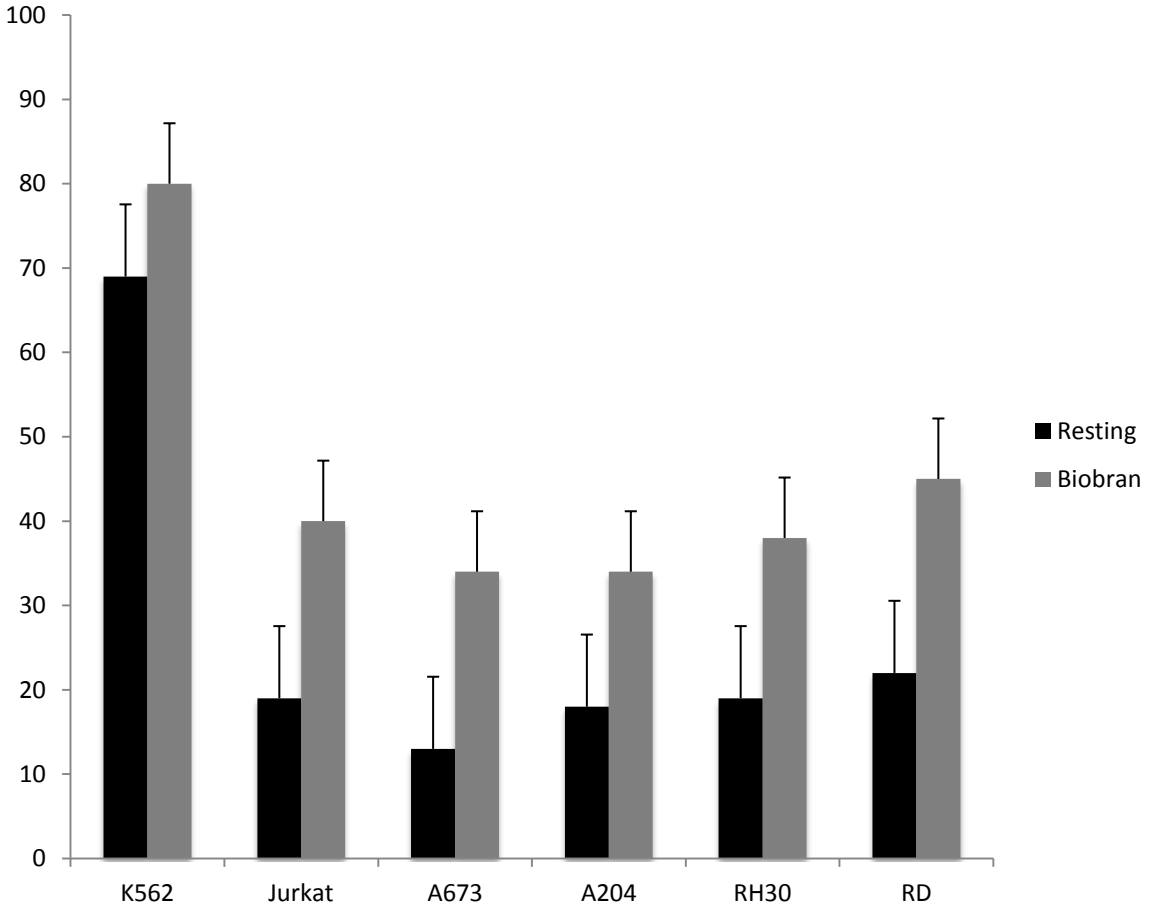
	20:1	10:1	5:1	2,5:1
ohne-IL	54	36	26	13
mit1000U-IL2	100	84	62	42
MGN-3 0,1mg/ml	77	56	40	24

NK Activity against Rhabdomyosarcoma

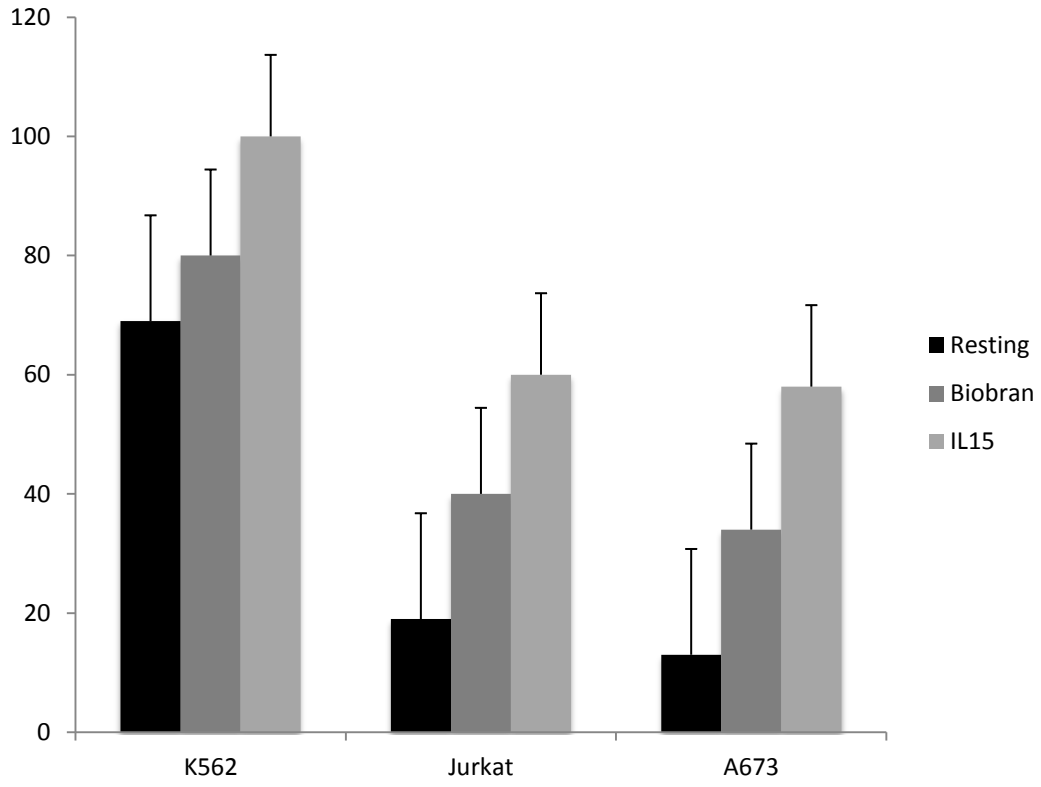


	20:1	10:1	5:1
nur NKC	25	15	15
40 U/ml IL2	62	36	28
0,1 mg/ml MGN3	47	27	14
40 U/ml IL2 + 0,1 mg/ml MGN3	100	62	39

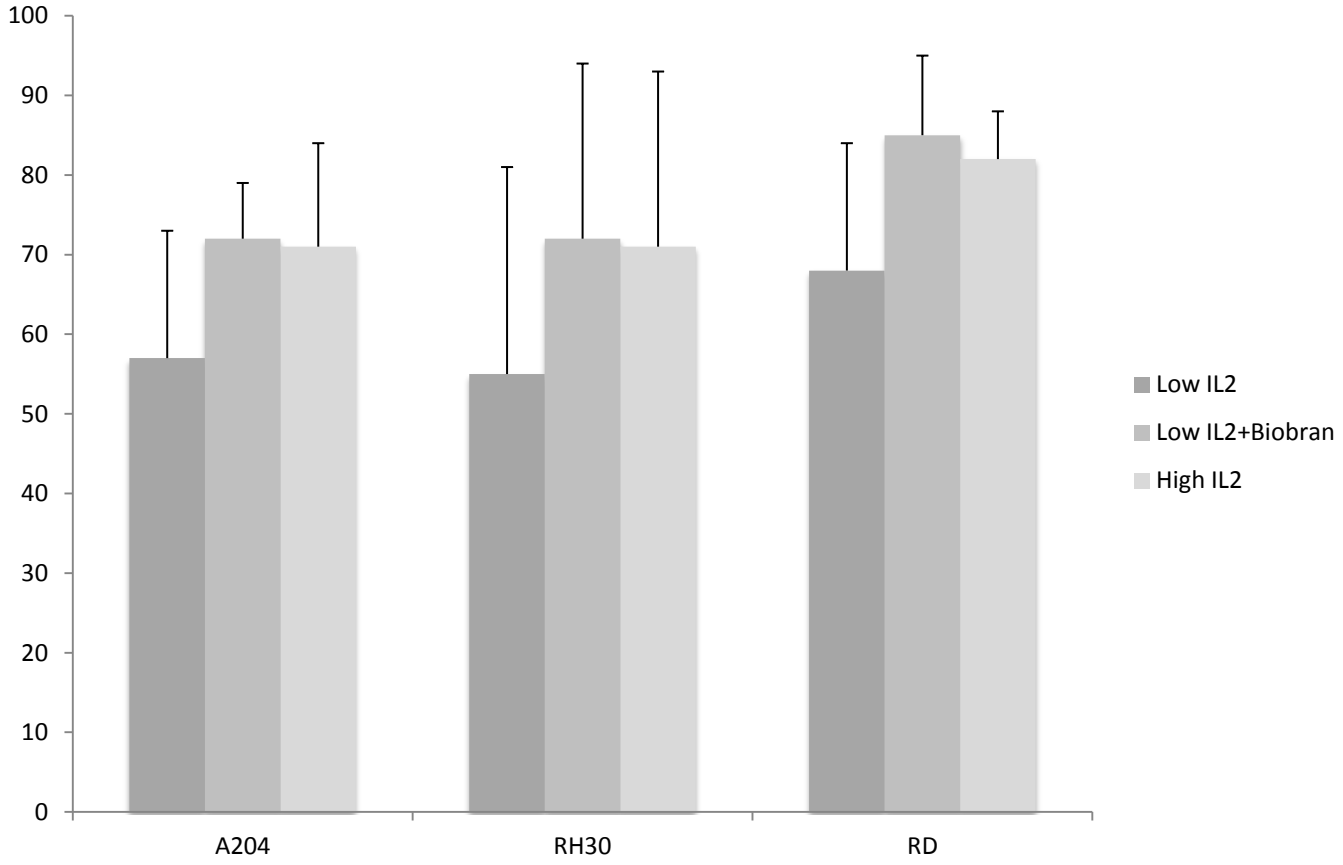
BioBran activated NK cell activity against various tumor cell lines



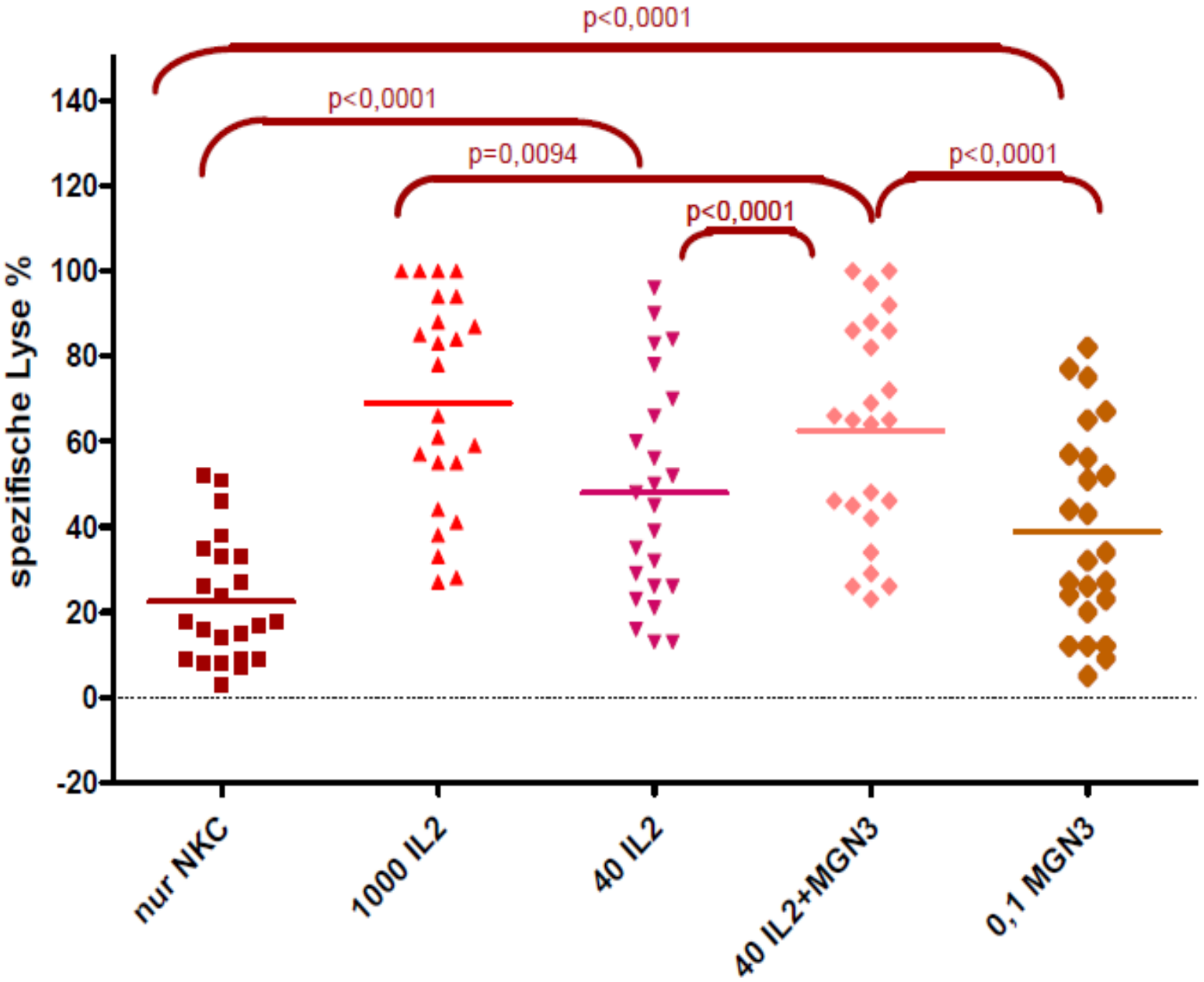
BioBran activated NK cell activity against various tumor cell lines



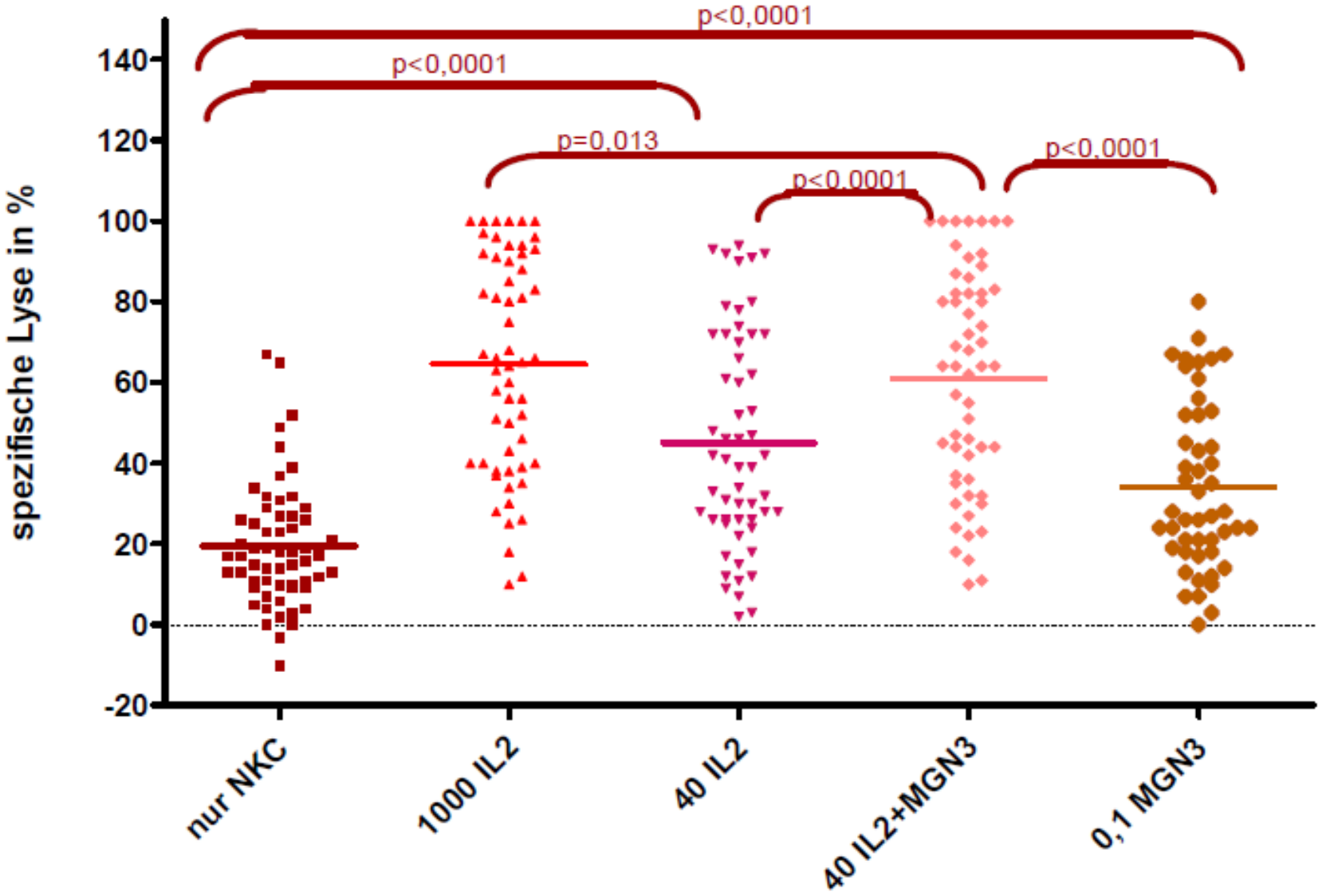
BioBran activated NK cell activity against various tumor cell lines



NK activity of healthy donors against K 562

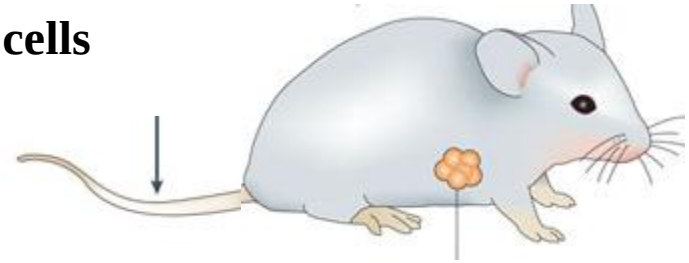


NK activity of healthy donors against RH30



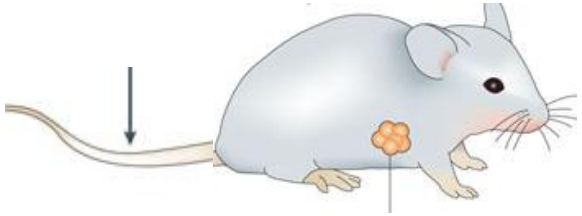
A xenograft model for neuroblastoma

Luciferase-marked NB cells

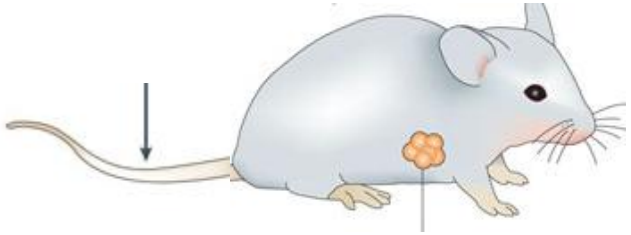


7 days

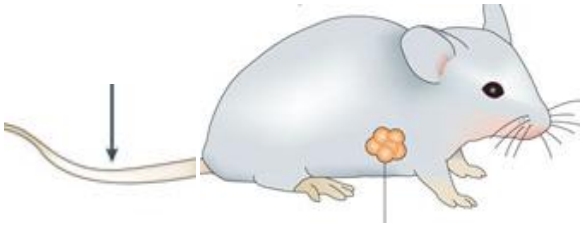
Saline



NK cells Medium

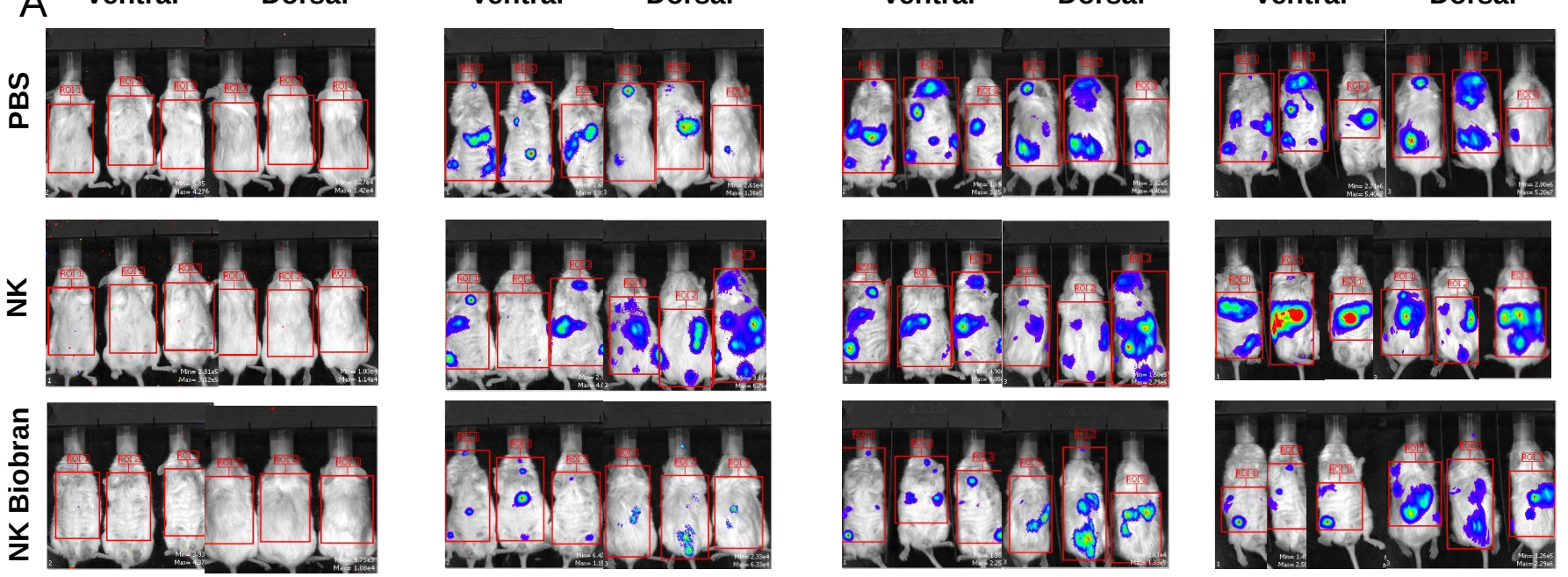


NK cells Activated overnight with BioBran

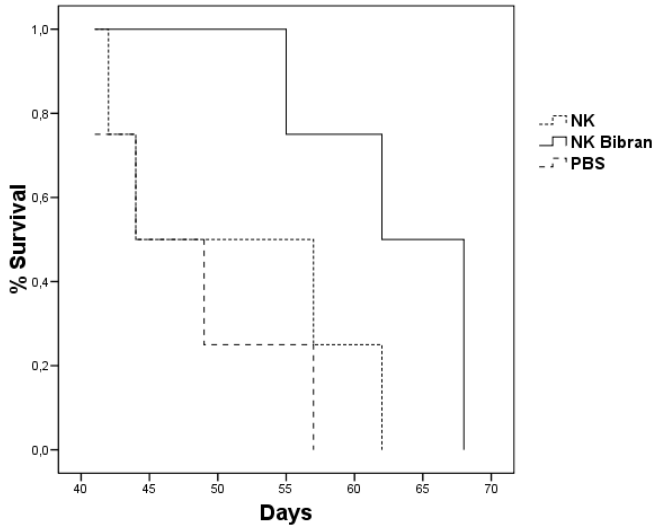


Treatment: twice weekly for 4 weeks

A

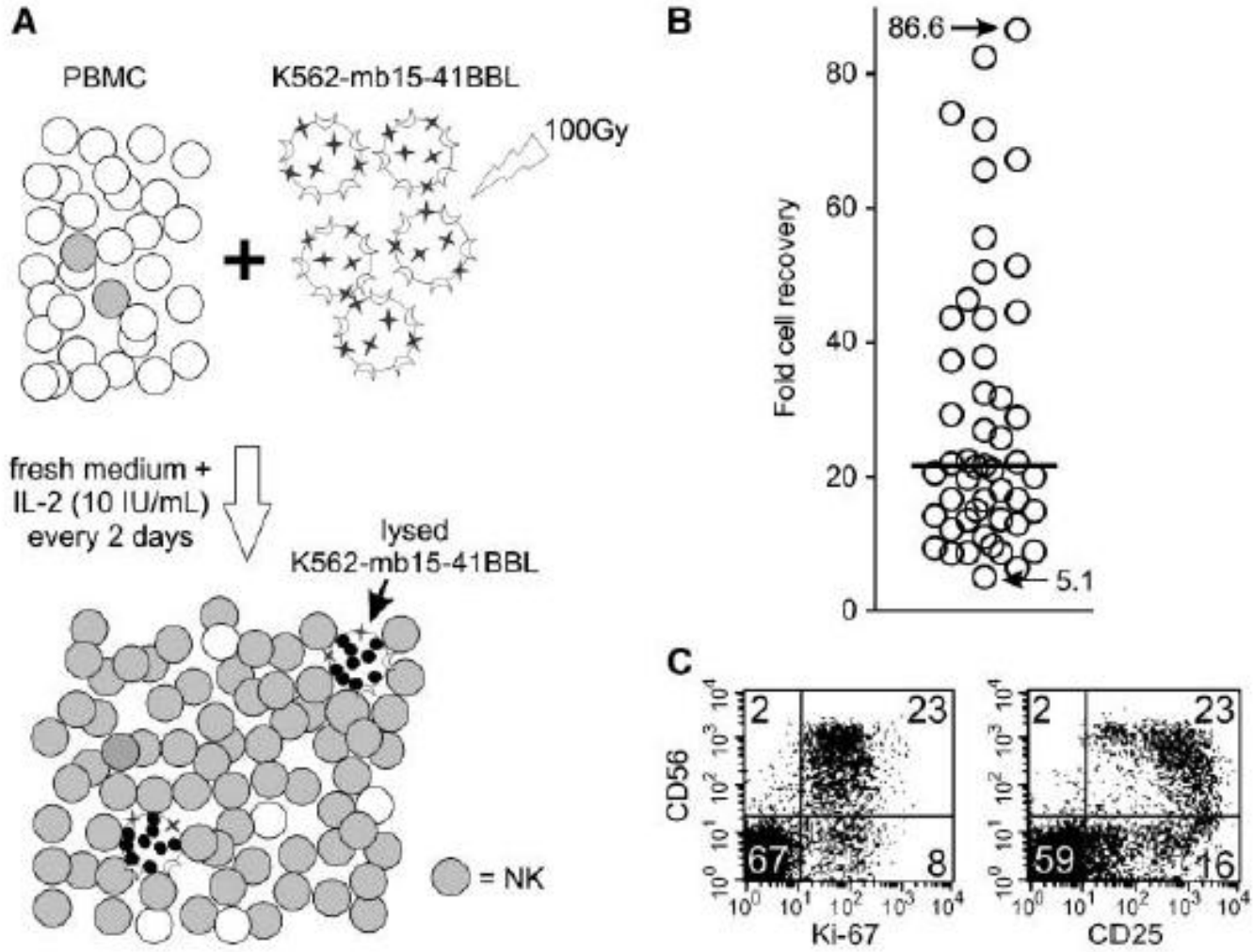

Ventral
Dorsal
Ventral
Dorsal
Ventral
Dorsal
Ventral
Dorsal
PBS
NK
NK Biobran
Day 7
Day 28
Day 35
Day 42

B

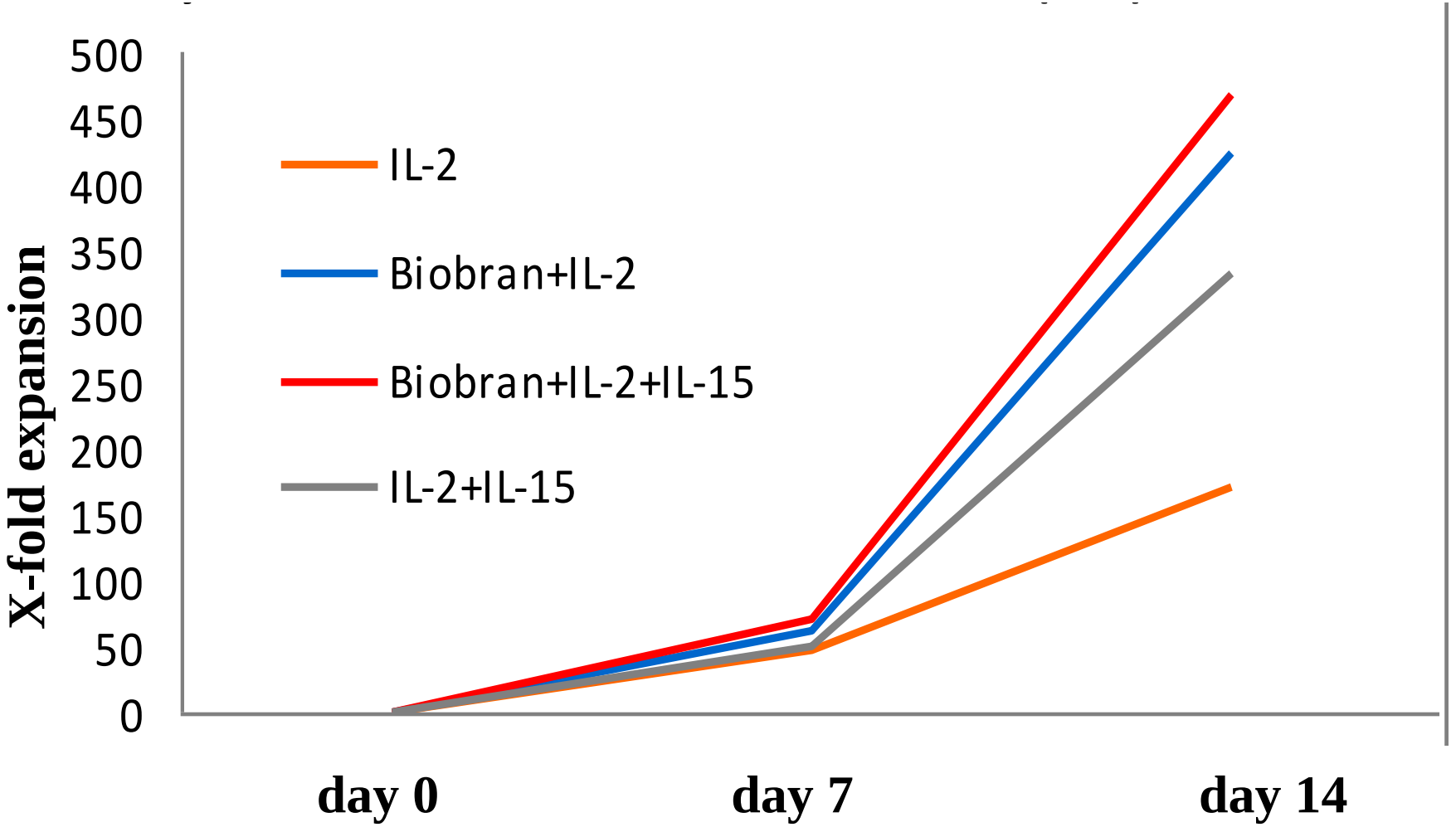

1,0
0,8
0,6
0,4
0,2
0,0
% Survival
40
45
50
55
60
65
70
Days
NK
NK Bibran
PBS

BioBran-stimulated in vitro expansion of NK cells

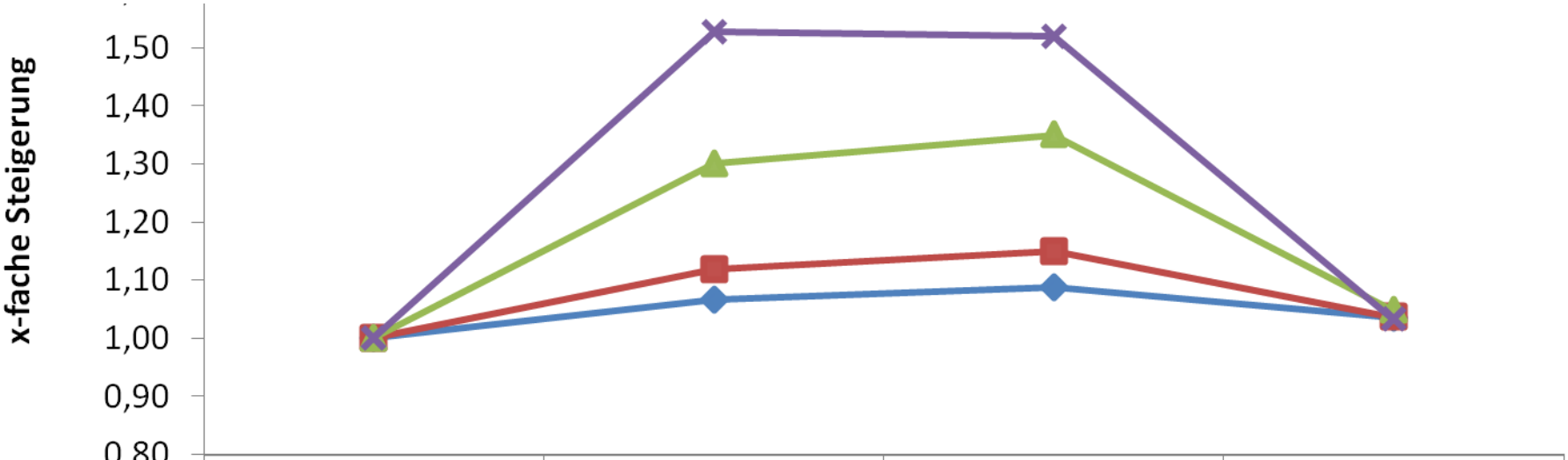
In vitro expansion von NK-Zellen
(Dr. Dario Campana, St.Jude Children‘s Research Hospital, Memphis, USA)



In vitro expansion of NK cells



NK activity of healthy individuals against K562 during Treatment with Biobran (n=10)



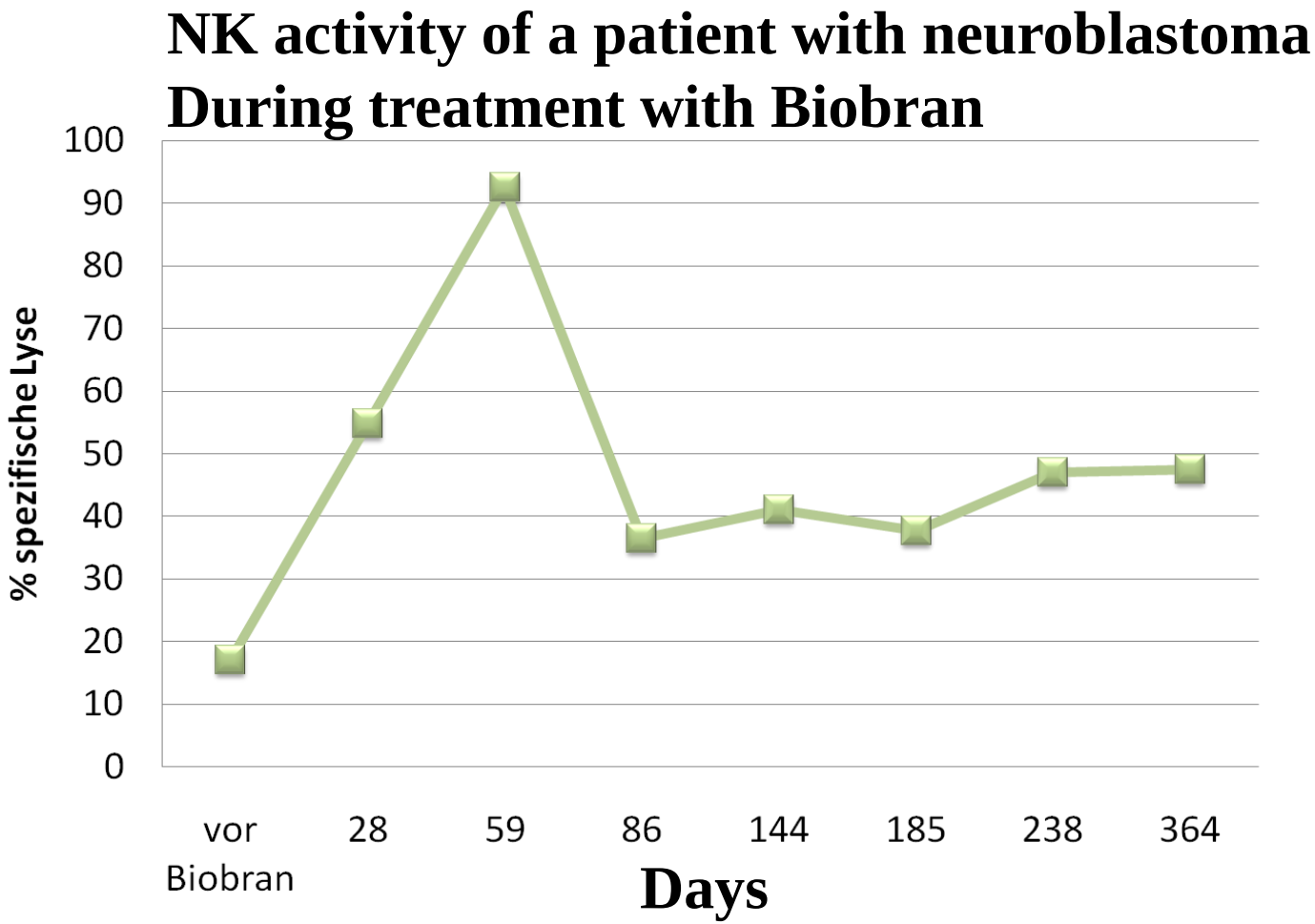
	vor Biobran	1 Woche Biobran	2 Wochen Biobran	2 Wochen nach Absetzen
20:1	1,00	1,07	1,09	1,04
10:1	1,00	1,12	1,15	1,04
5:1	1,00	1,30	1,35	1,05
2,5:1	1,00	1,53	1,52	1,03

NK-Activity of patients

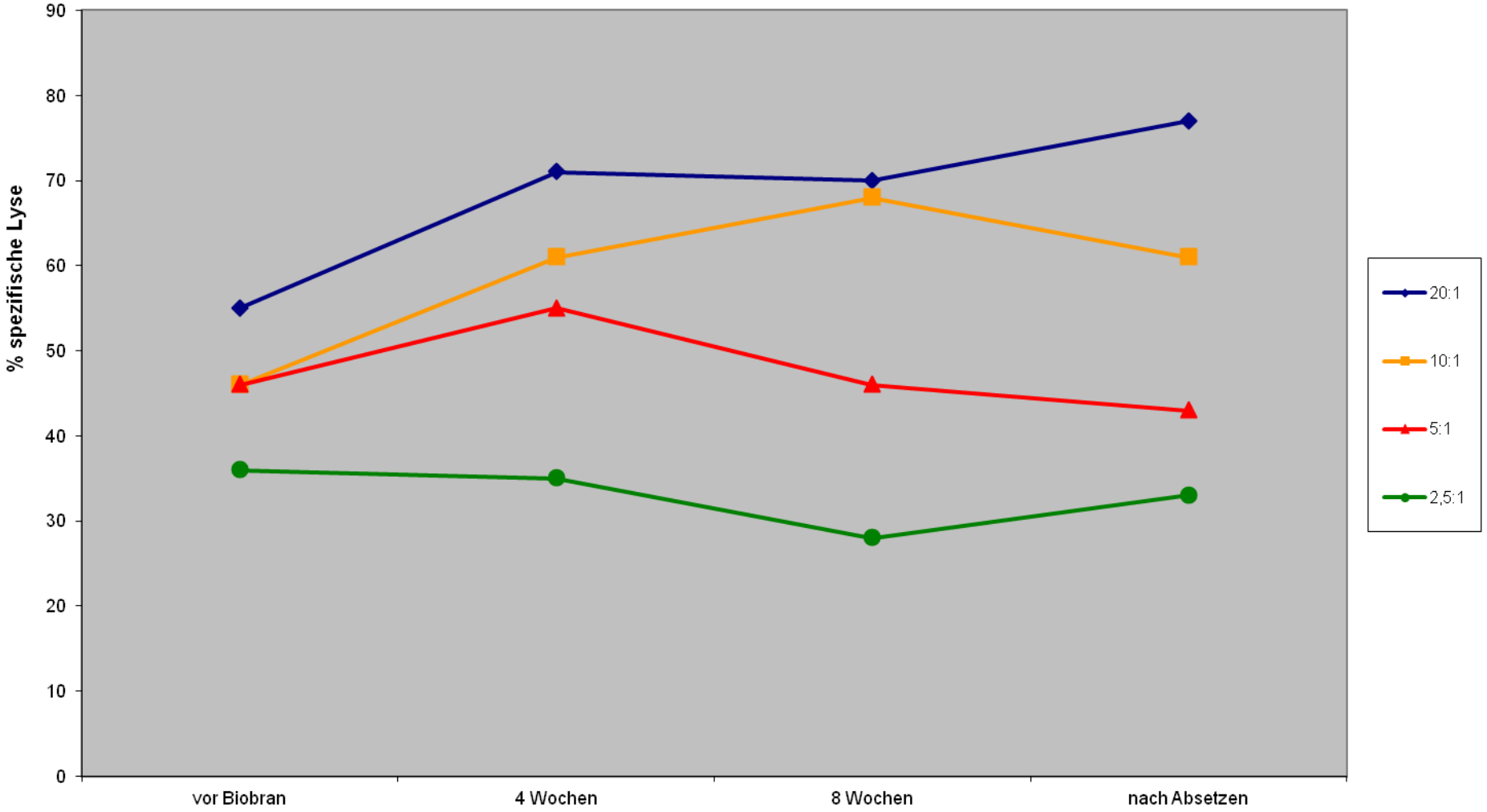
- 10 patients with sarcomas were treated
- 6/10 showed increase of their NK activity

Protokol for pediatric patients with various malignancies

Biobran 3 x 2 sachets p.o /day (plus Interleukin2 s.c.)



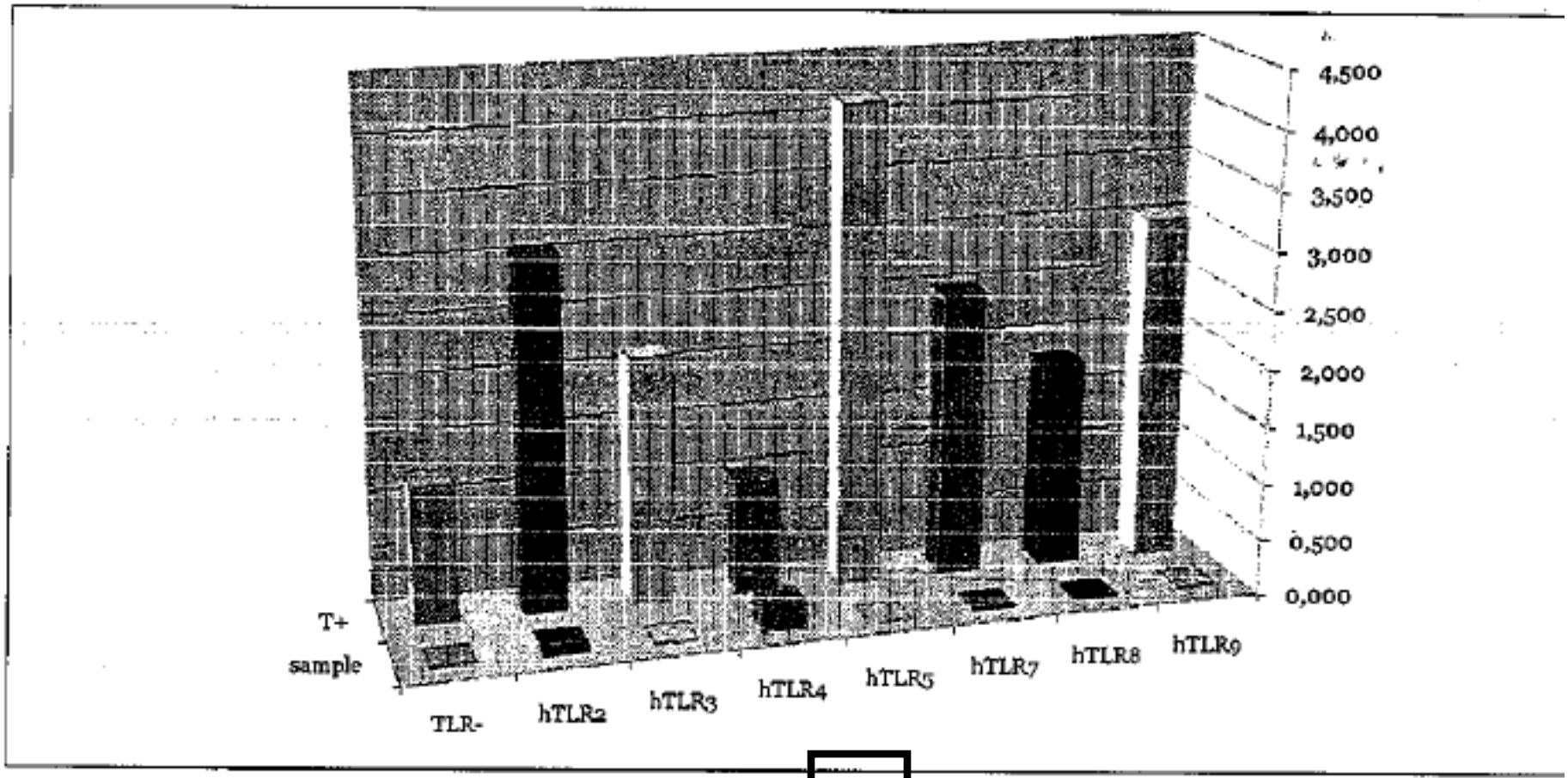
Patient


% spezifische Lyse
90
80
70
60
50
40
30
20
10
0
vor Biobran
4 Wochen
8 Wochen
nach Absetzen
20:1
10:1
5:1
2,5:1

Patient

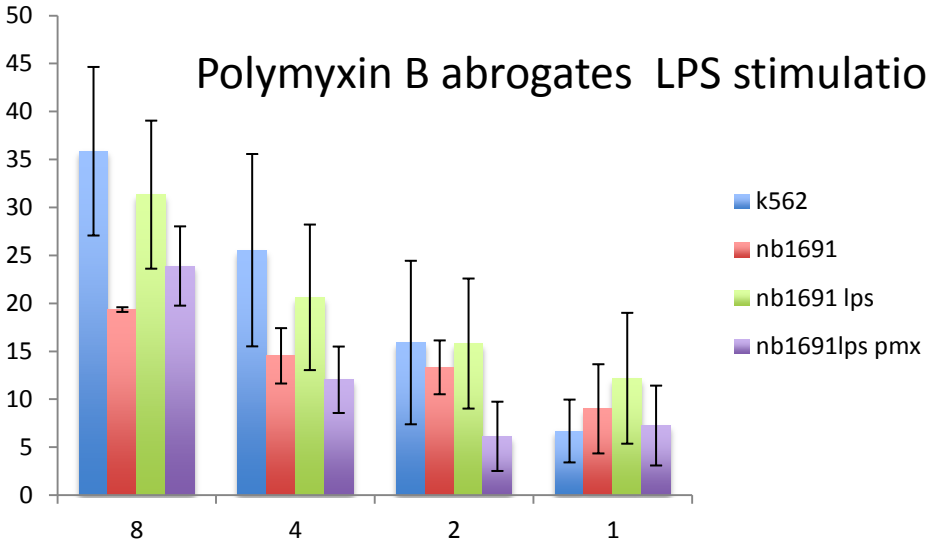
	vor Biobran	14 Tage Biobran	28 Tage Biobran	14 Tage IL-2	14 Tage IL2+Biobran
K562	-1	15	13	6	21
A 673	-8	31	21	13	18

The potential role of Lipopolysaccharide (LPS)

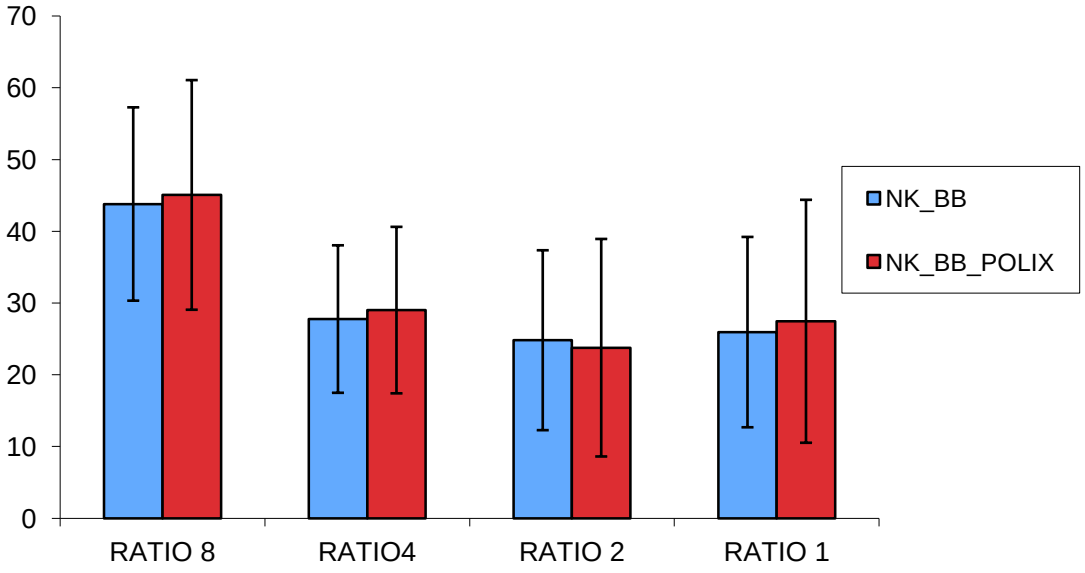


	TLR-	hTLR2	hTLR3	hTLR4	hTLR5	hTLR7	hTLR8	hTLR9
T+	1,233	3,058	2,168	1,012	4,173	2,501	1,818	3,043
sample	0,032	0,080	0,020	0,245	-0,018	0,024	0,020	0,038

The role of LPS contamination of Biobran



Polymyxin B does not abrogate Biobran-activated NK cell cytotoxicity



Questions?

- What is the molecular mechanism how BioBran activates NK cells?
- What is the dose at which NK cells are optimally activated in vivo?
- Do combinations of Biobran with other cytokines (i.e. Interleukin 2, Interleukin 15) have synergistic effects?