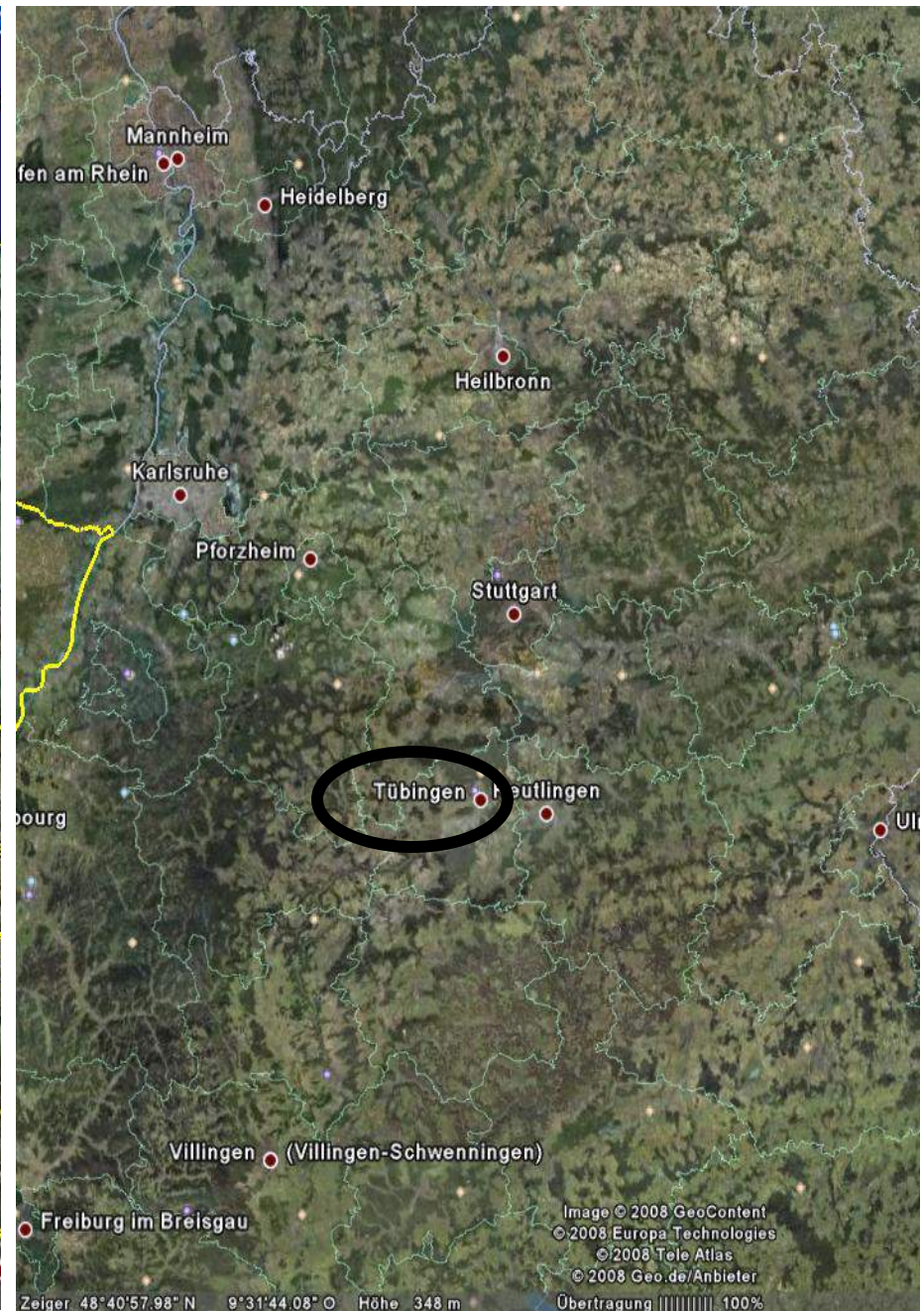
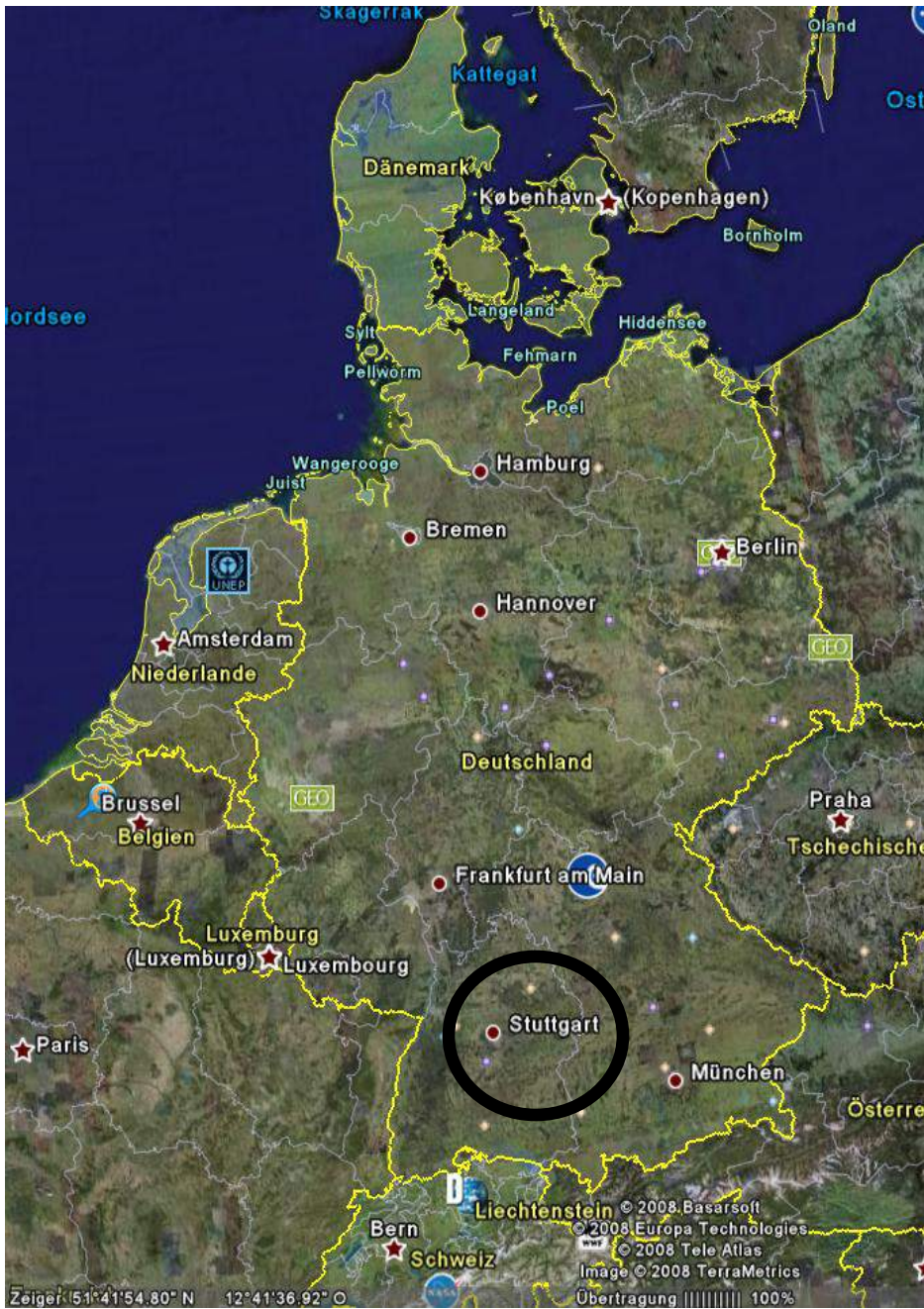


The role of BioBran in NK-cell mediated treatment strategies in pediatric cancer

Rupert Handgretinger

Children's University Hospital
Tübingen, Germany





City Hall



Bursa (1480)



Stift (1260)



Major: B. Palmer
Green Party

1514: P. Melancthon, Reformer
1808: University Hospital

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

Catholic faculty



Castle Hohentübingen



Pope Benedict (1966-69)
Prof. Hans Küng

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)

C'est dans l'ancienne cuisine du château de Hohentübingen, que Friedrich Miescher, chercheur originaire de Bâle, découvre en 1869 la substance acide contenue dans le noyau des cellules humaines.

Cette substance, qu'il appela nucléine, contient l'ADN, support de l'hérédité et fondement de la biologie et de la médecine moléculaire.

In 1869, in the former kitchen here in the Castle of Hohentübingen, Friedrich Miescher from Basel isolated an acidic substance from the nuclei of human cells.

This substance, "Nuklein", contains DNA that is now known to be the carrier of genetic information.

This discovery proved to be the foundation of molecular biology and medicine.

Erwin Bälz, MD (1849-1913)

1876-1905: Reformer of Japanese medicine

Official physician to emperor Meiji



Gedächtnis- und Keisai-Denkmal für den Arzt Erwin Bälz
geboren am 13. 01. 1849 in Bielefeld
1876 - 1905 Studium in Tokio
1876 - 1905 Stellvertreter des japanischen Medizins- und
Leibkunds des Kaisers von Japan
Die Internationale Medizinische Gesellschaft Japans
erhielt ihn mit der Inschrift:
"Erwin Bälz, Du hast die japanische Medizin
zu ihrer ersten Blüte erwacht."
(Shiroshi Maehara)

Erwin Bälz,
you have awakened Japanese medicine
to its first blossoming.

(International 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.



FIG. II.—DR. COLEY'S CASE OF SARCOMA OF LOWER END OF FEMUR.
FIGURE SHOWING GRANULATING SURFACES PRODUCED BY
THE EFFUSION OF COLEY.

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

Immune system

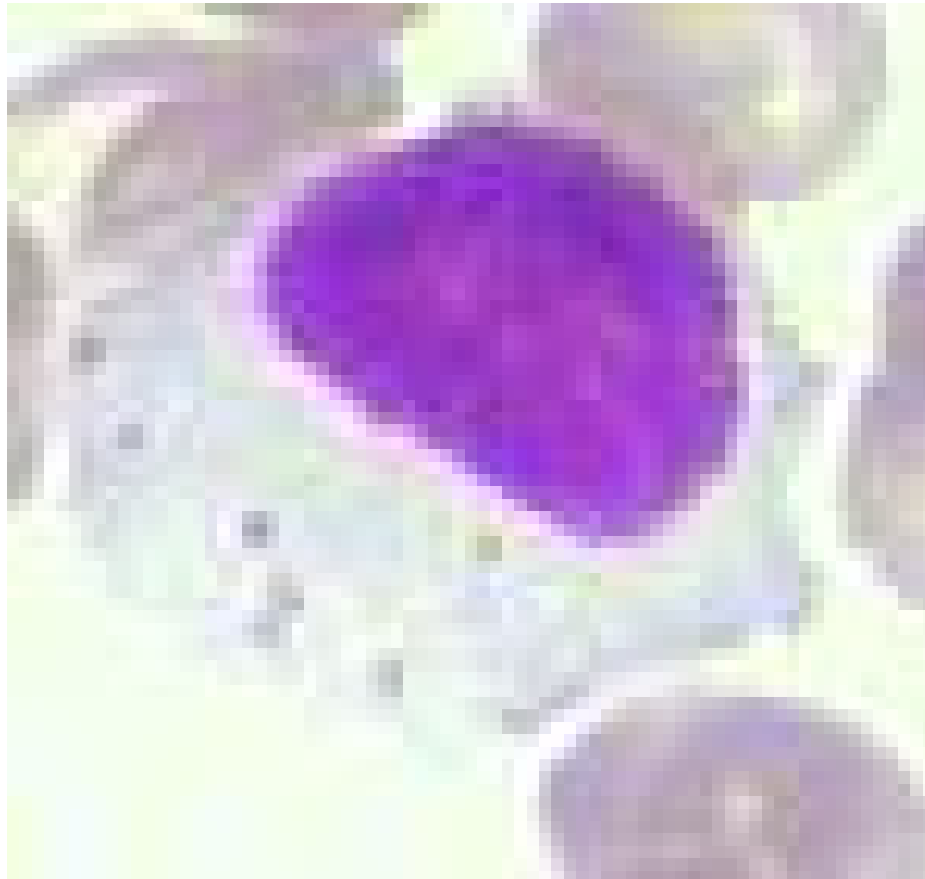
Innate immune system

Adaptive immune system

Natural Killer (NK)cells

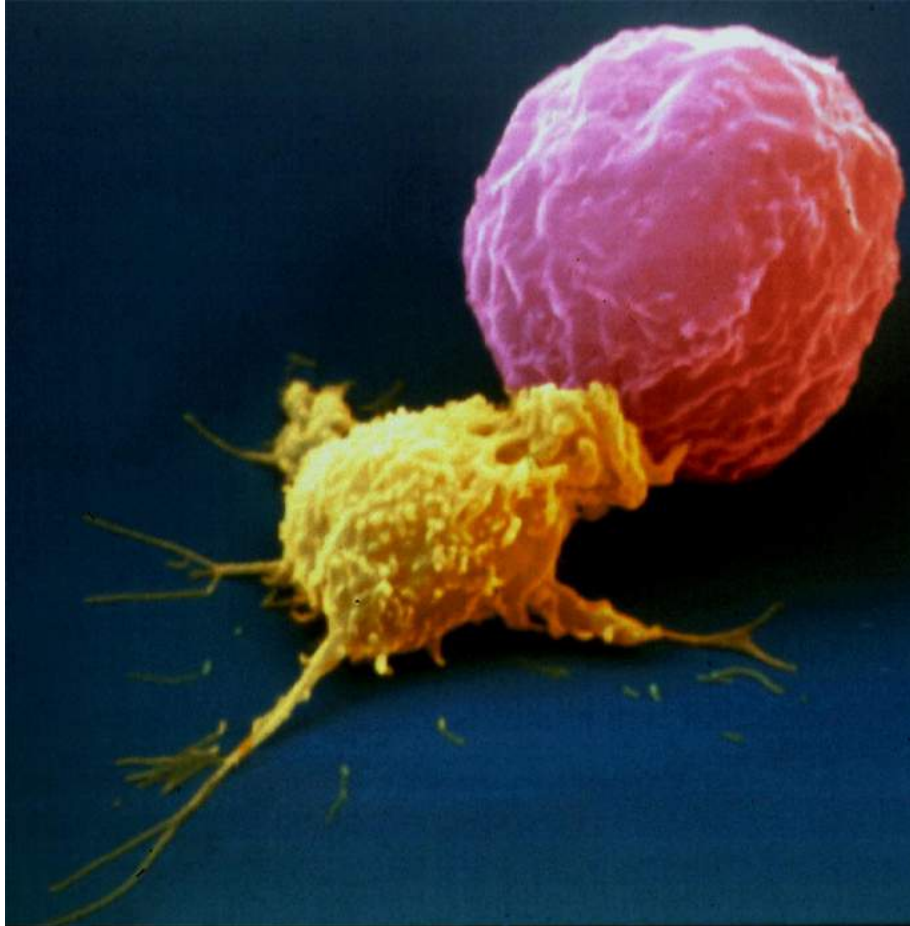
T-lymphocyte system

Natural Killer (NK) cells



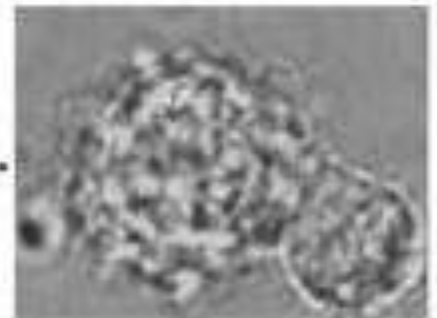
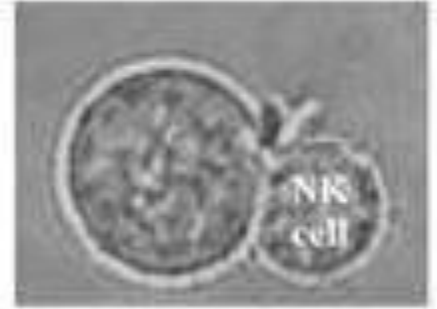
NK cell attacks a leukemic blast (K562 Erythroleukemia)

NK Assay



Eyeofscience, Reutlingen

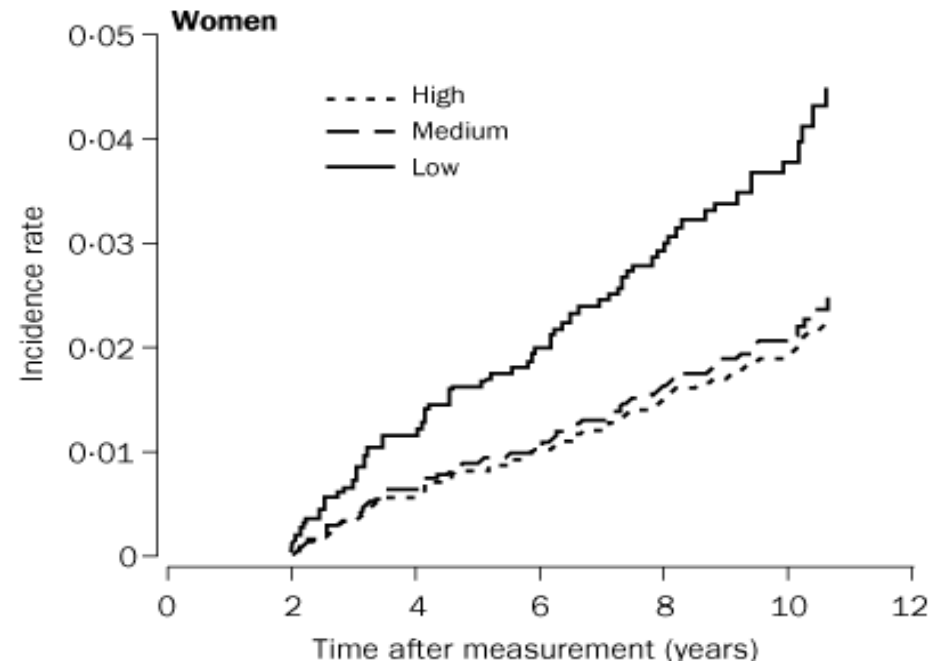
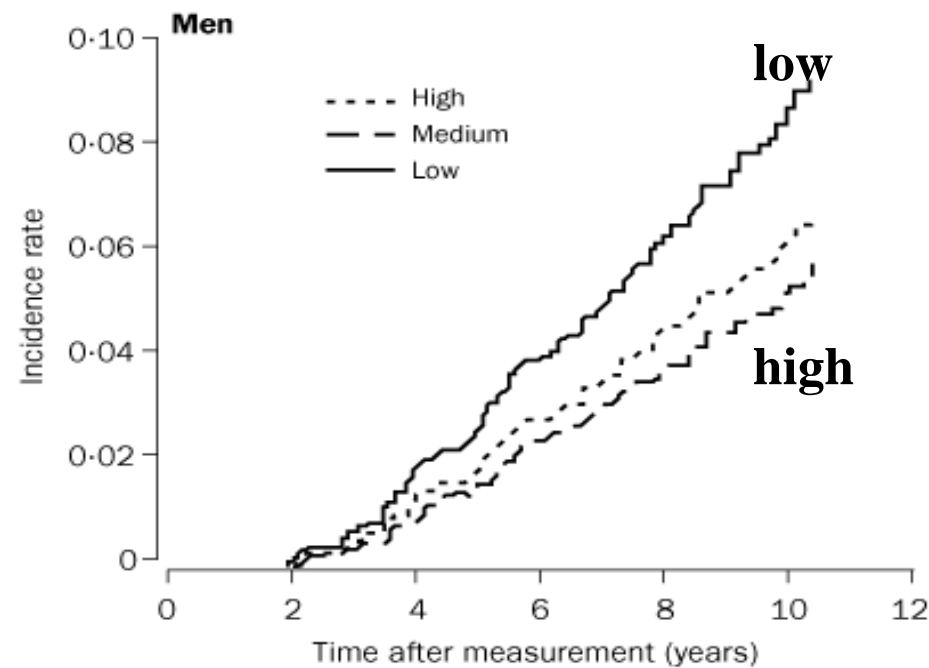
Natural Killer (NK) cells lyse cells that are deficient in expression of class I MHC proteins



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

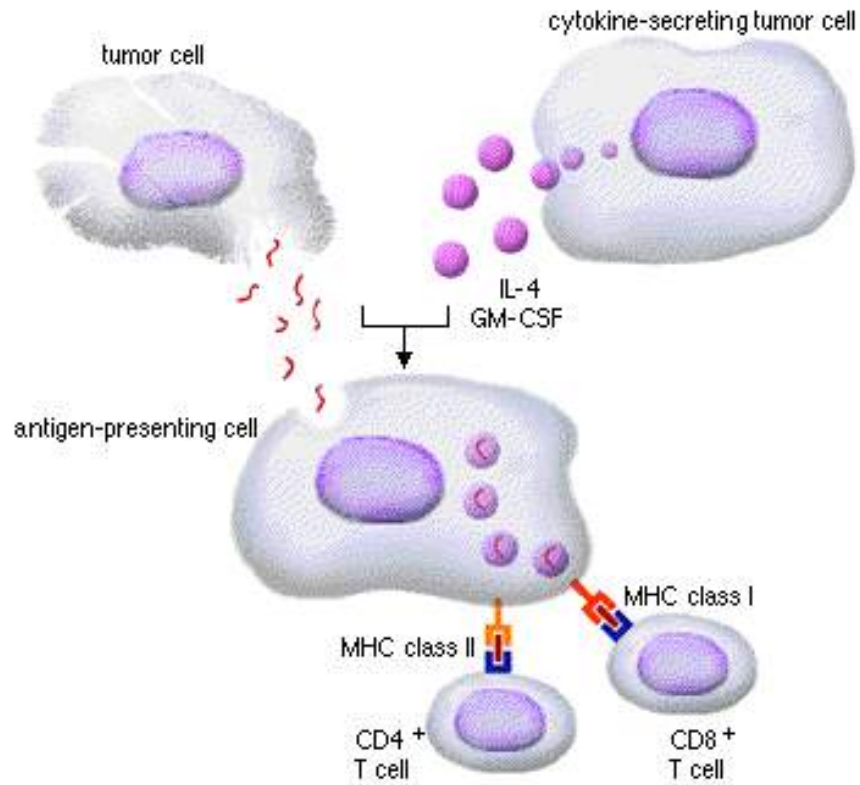
In 1982, Sweden claimed that it had recordings from a Soviet submarine in the waters of the Stockholm archipelago. The recording was made during a submarine hunt by the Swedish military on October 12th, 1982. It strengthened suspicions that Soviet subs were intruding in Swedish waters.

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

A

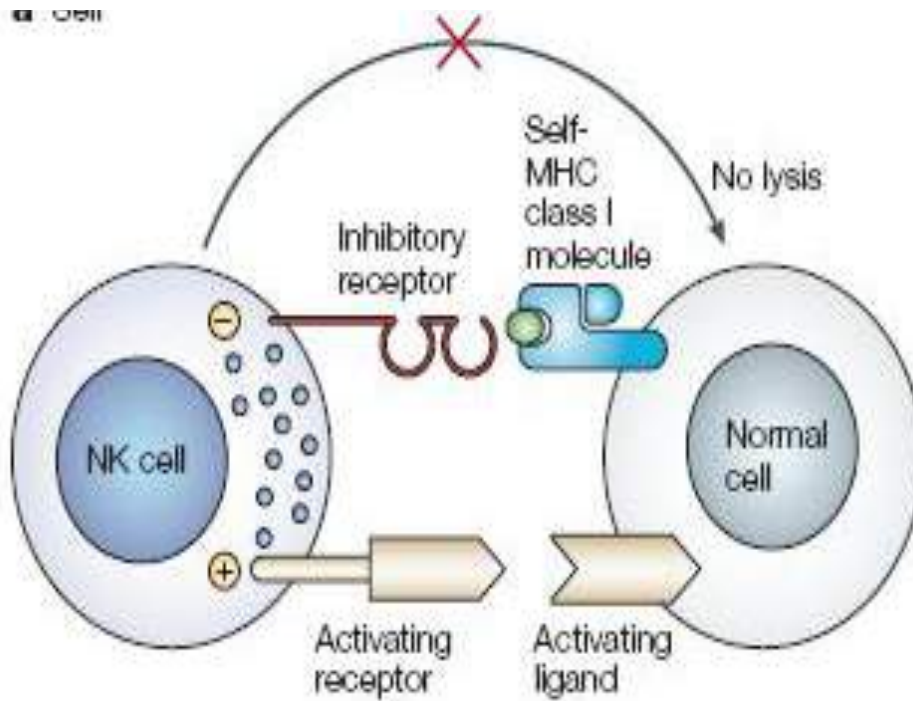


B

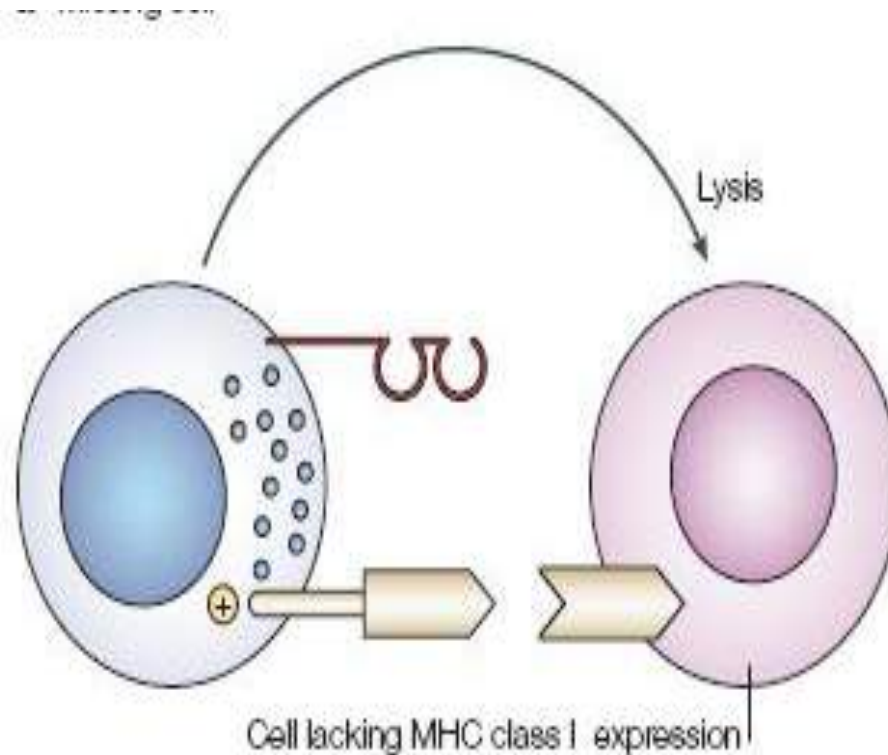


Nk cells have inhibitory receptors which recognize HLA class I antigens

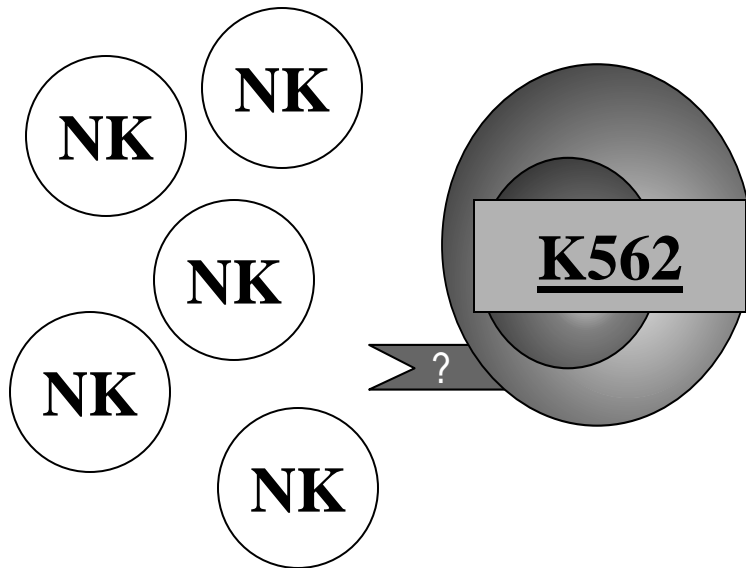
Do not call the marine



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



Why are K 562 good target cells?

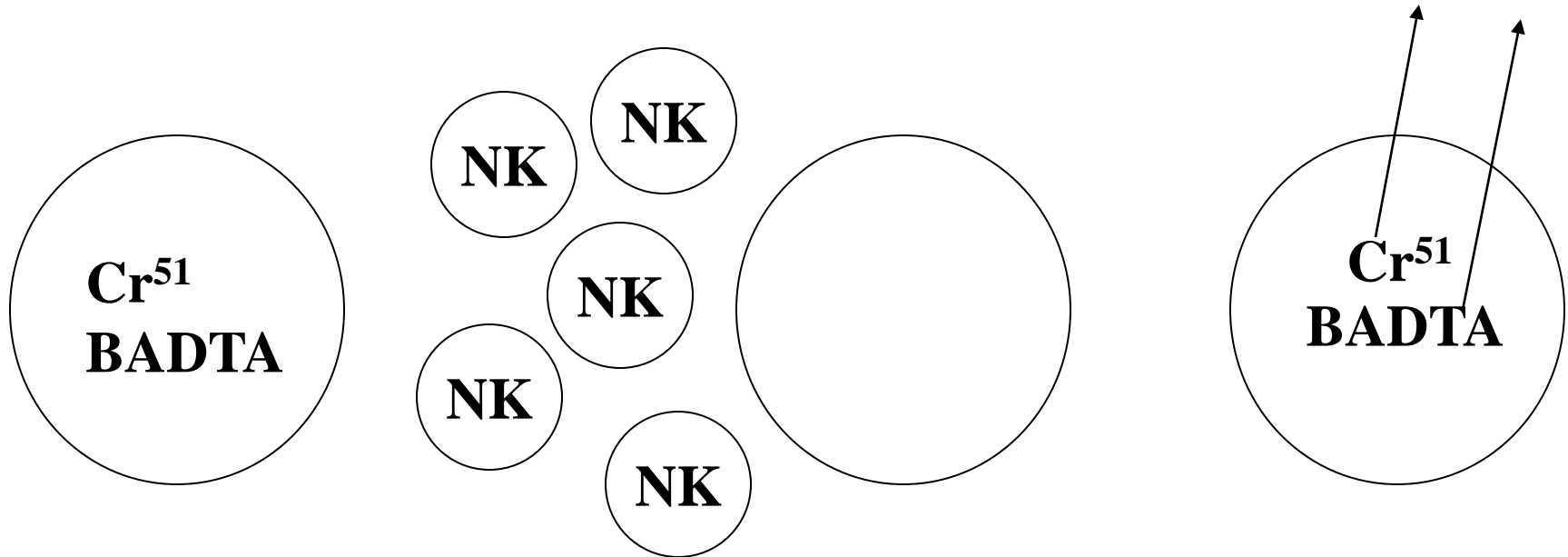


K562 (Erythroleukämie):

They lack HLA class I

How to measure NK activity?

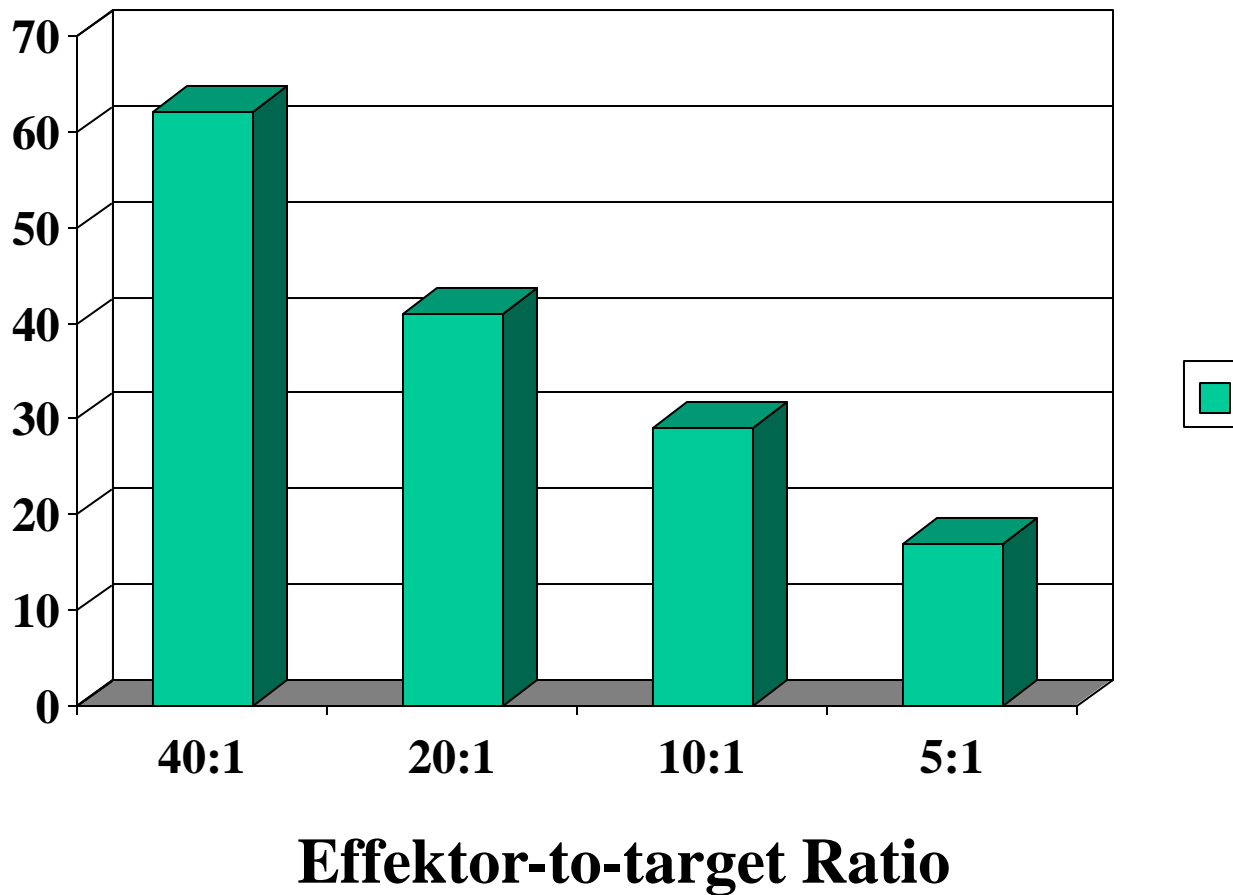
K562 Erythroleukämie



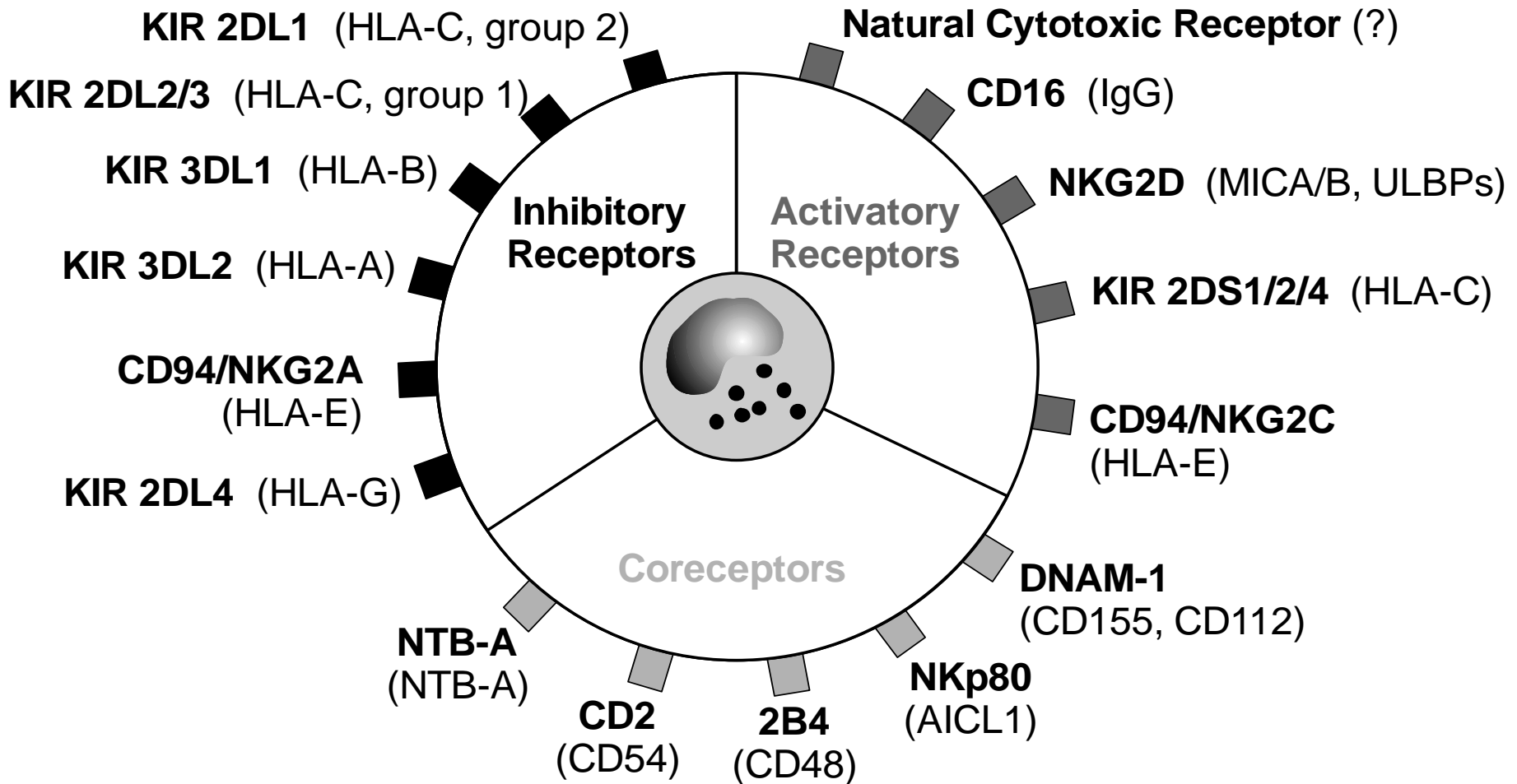
Effektor-to-target ratio

Cytotoxicity

Specific lysis

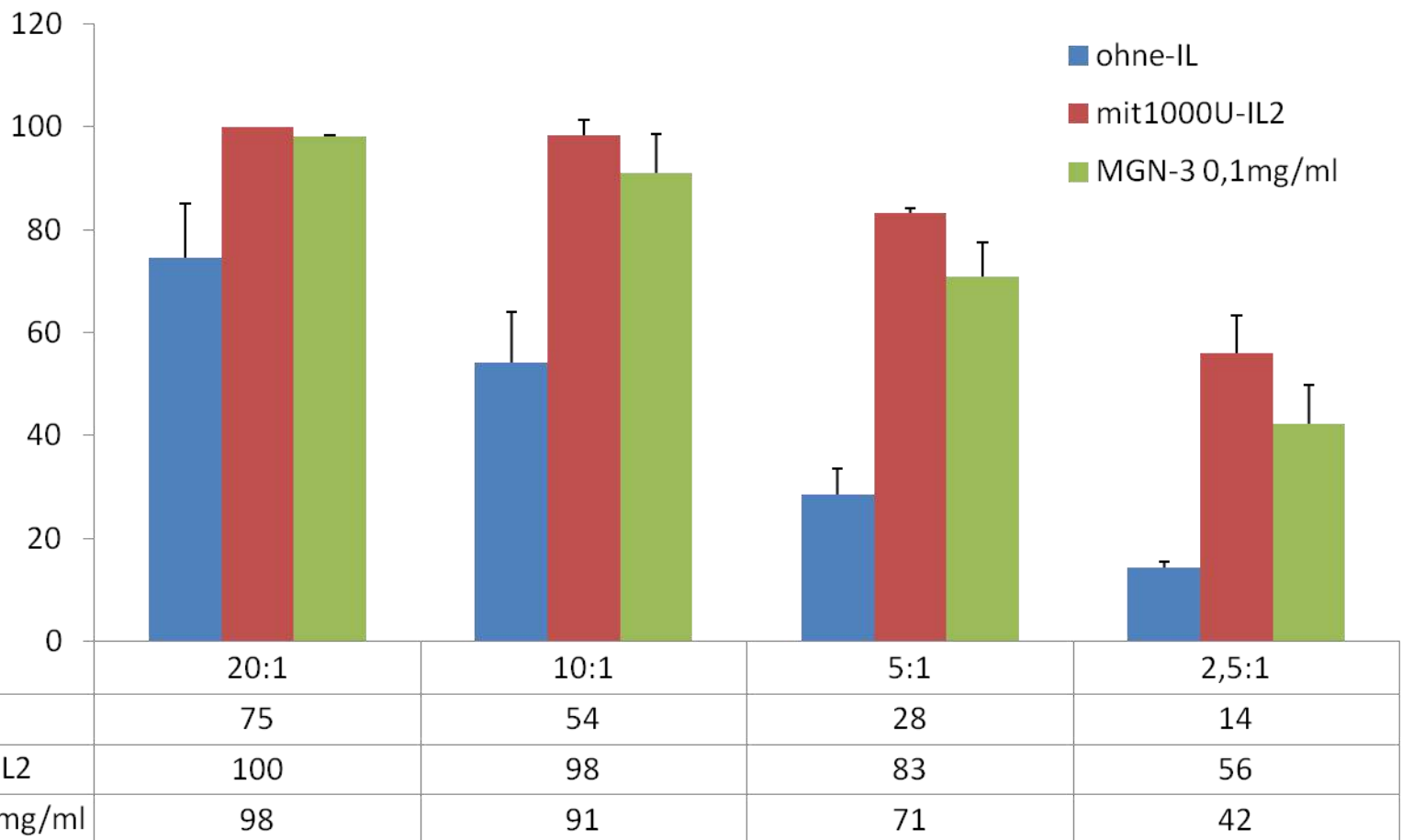


Inhibitory, activatory and Coreceptors on NK cells

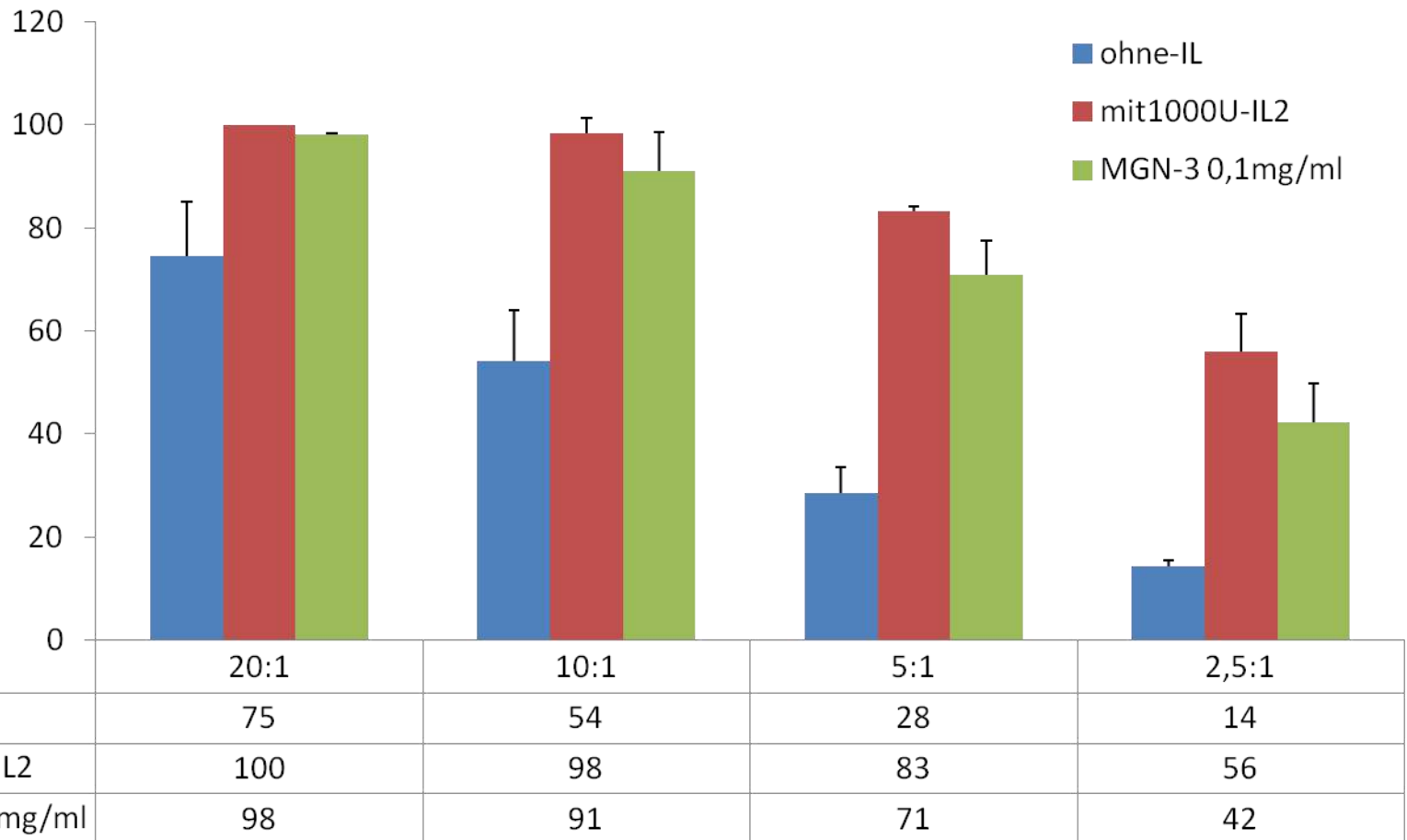


BioBran activates NK cells in vitro

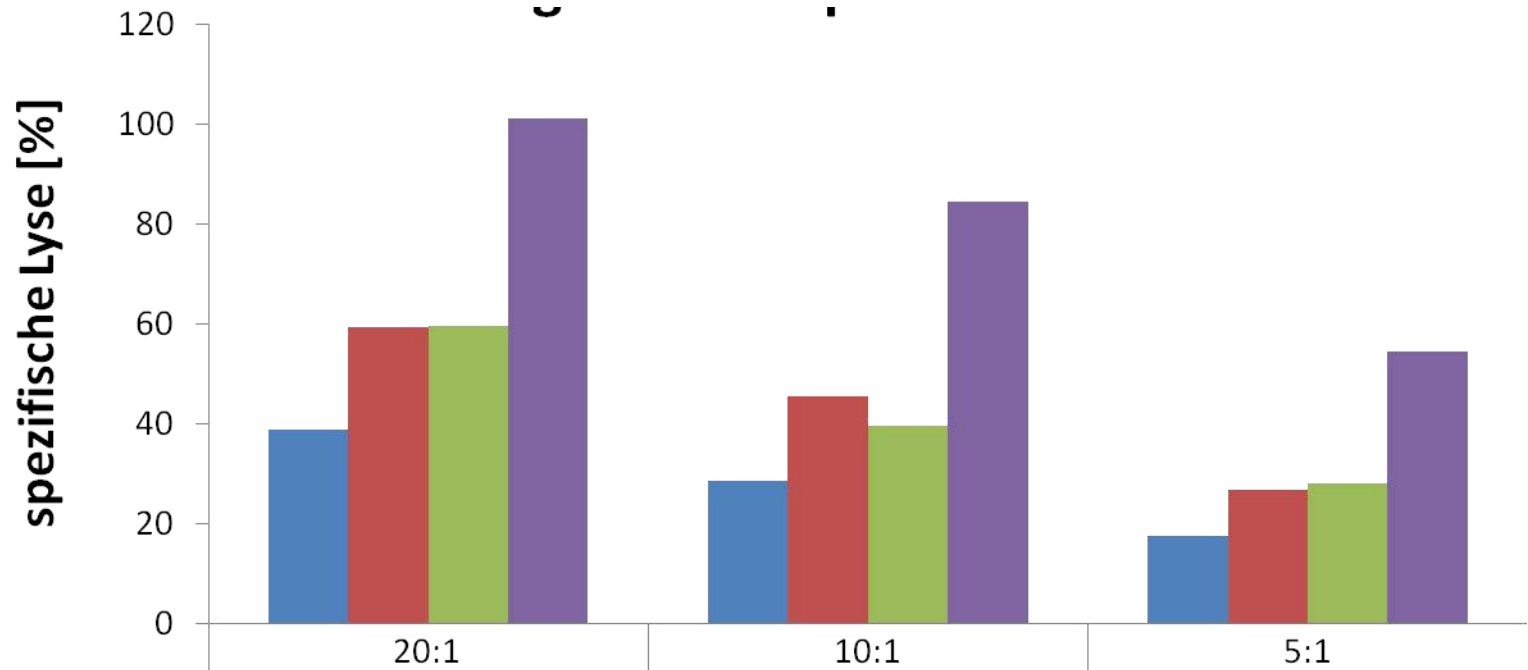
NK Activity of healthy donors against K 562



NK Activity of healthy donors against K 562

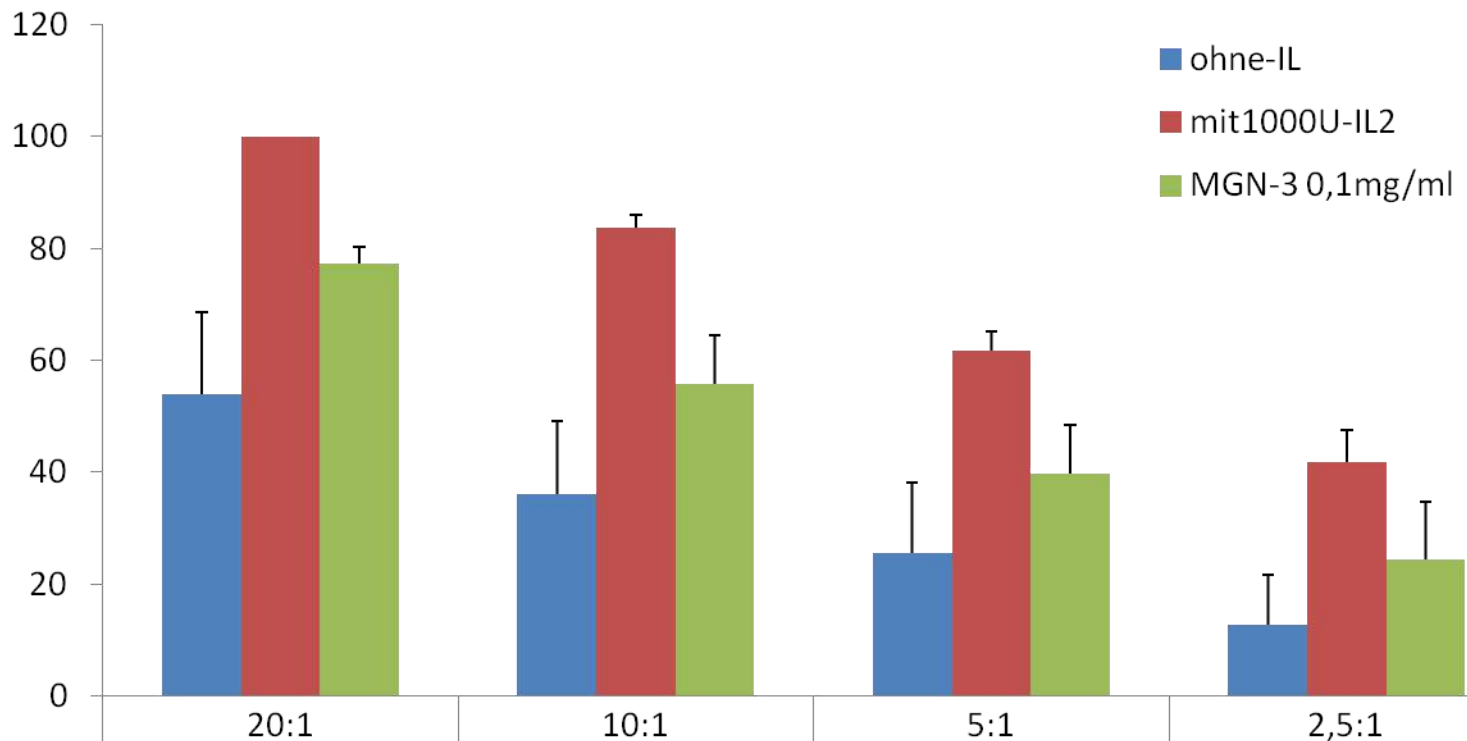


NK Activity of healthy donors against K 562



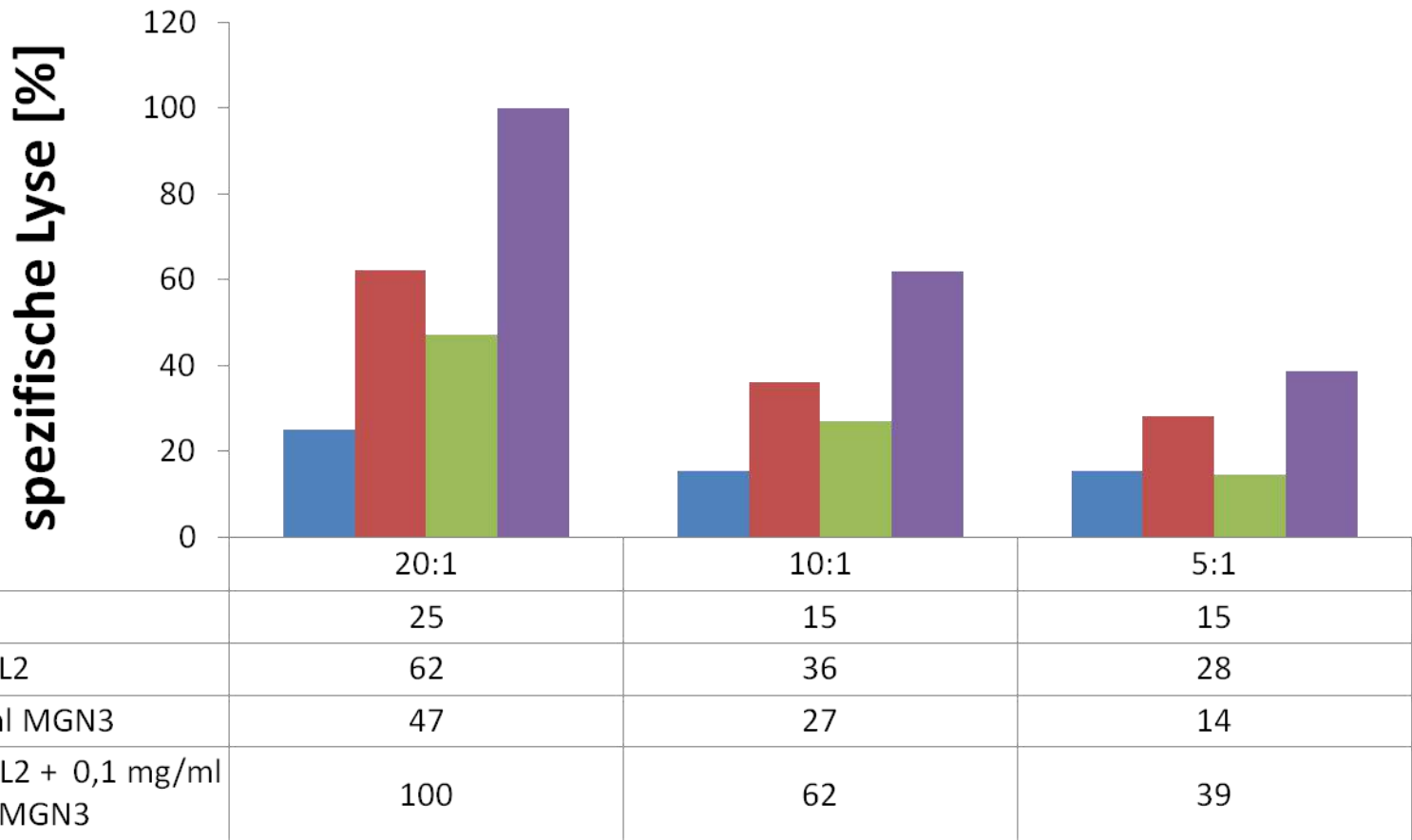
	20:1	10:1	5:1
■ nur NKC	39	29	17
■ 40 U/ml IL2	59	46	27
■ 0,1 mg/ml MGN3	60	39	28
■ 40 U/ml IL2 + 0,1 mg/ml MGN3	101	85	54

NK Activity against Hepatoblastoma

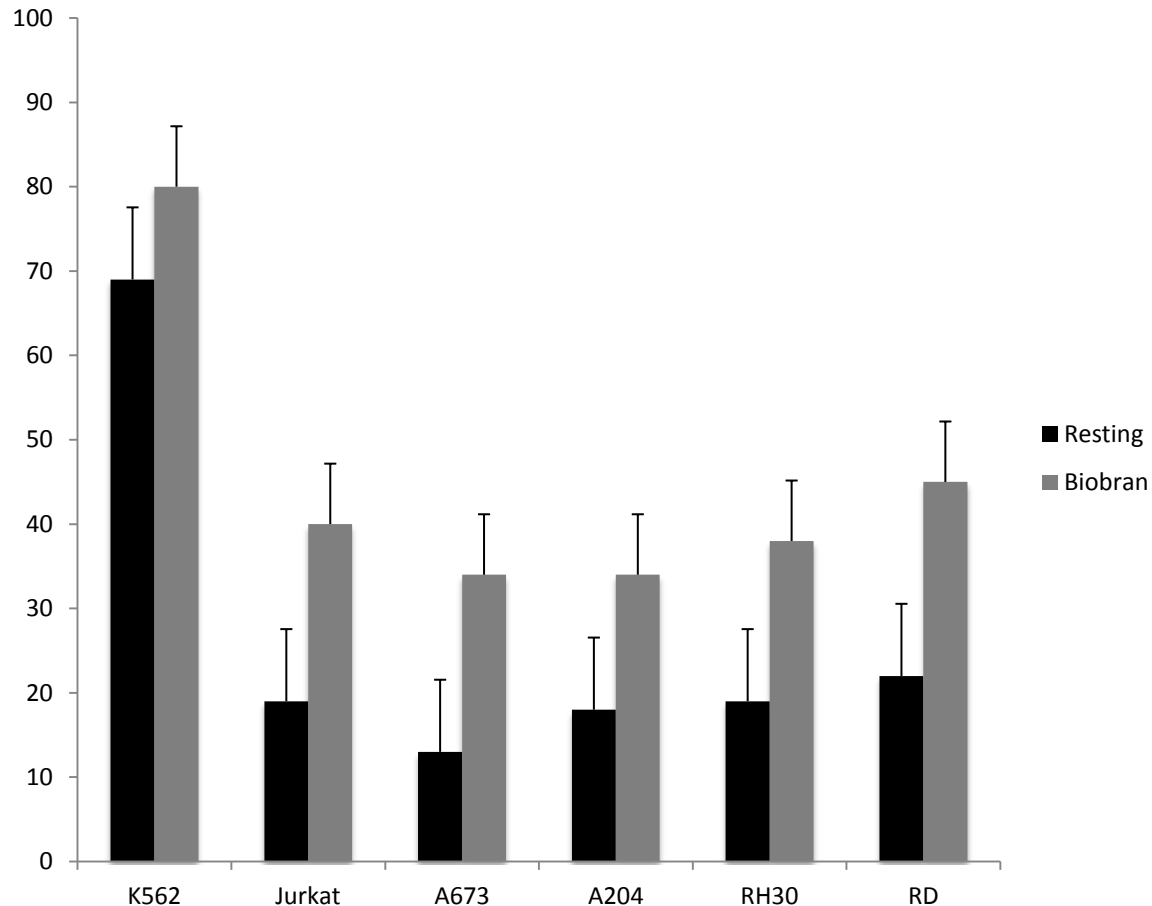


ohne-IL	54	36	26	13
mit1000U-IL2	100	84	62	42
MGN-3 0,1mg/ml	77	56	40	24

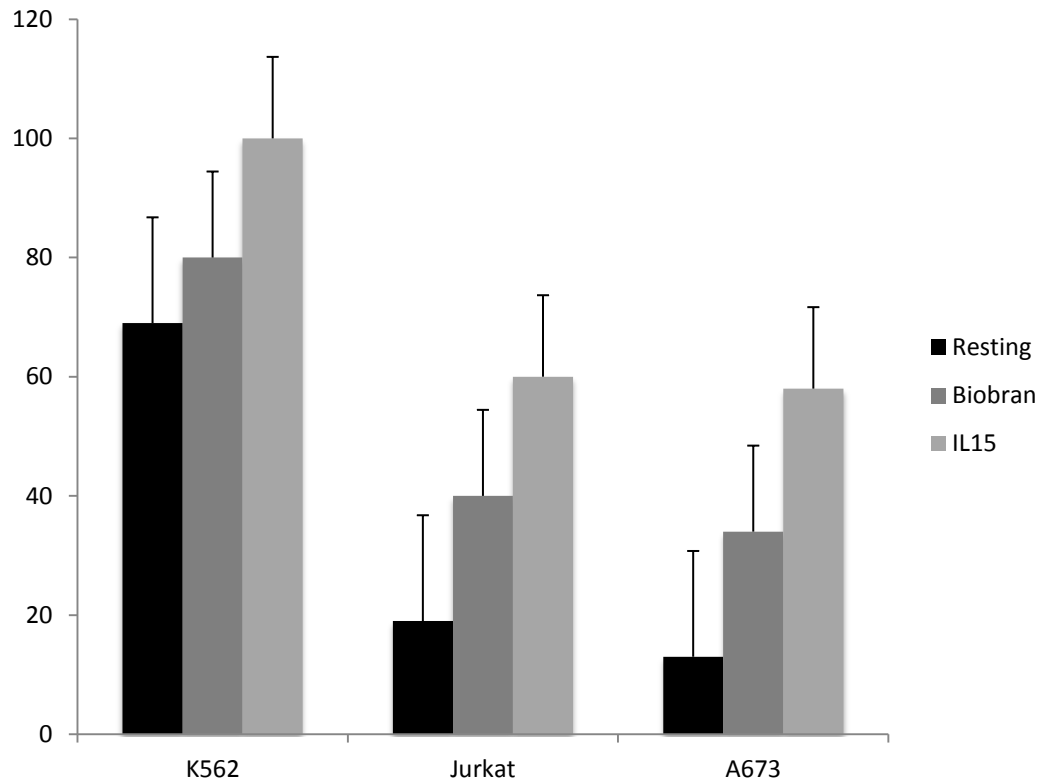
NK Activity against Rhabdomyosarcoma



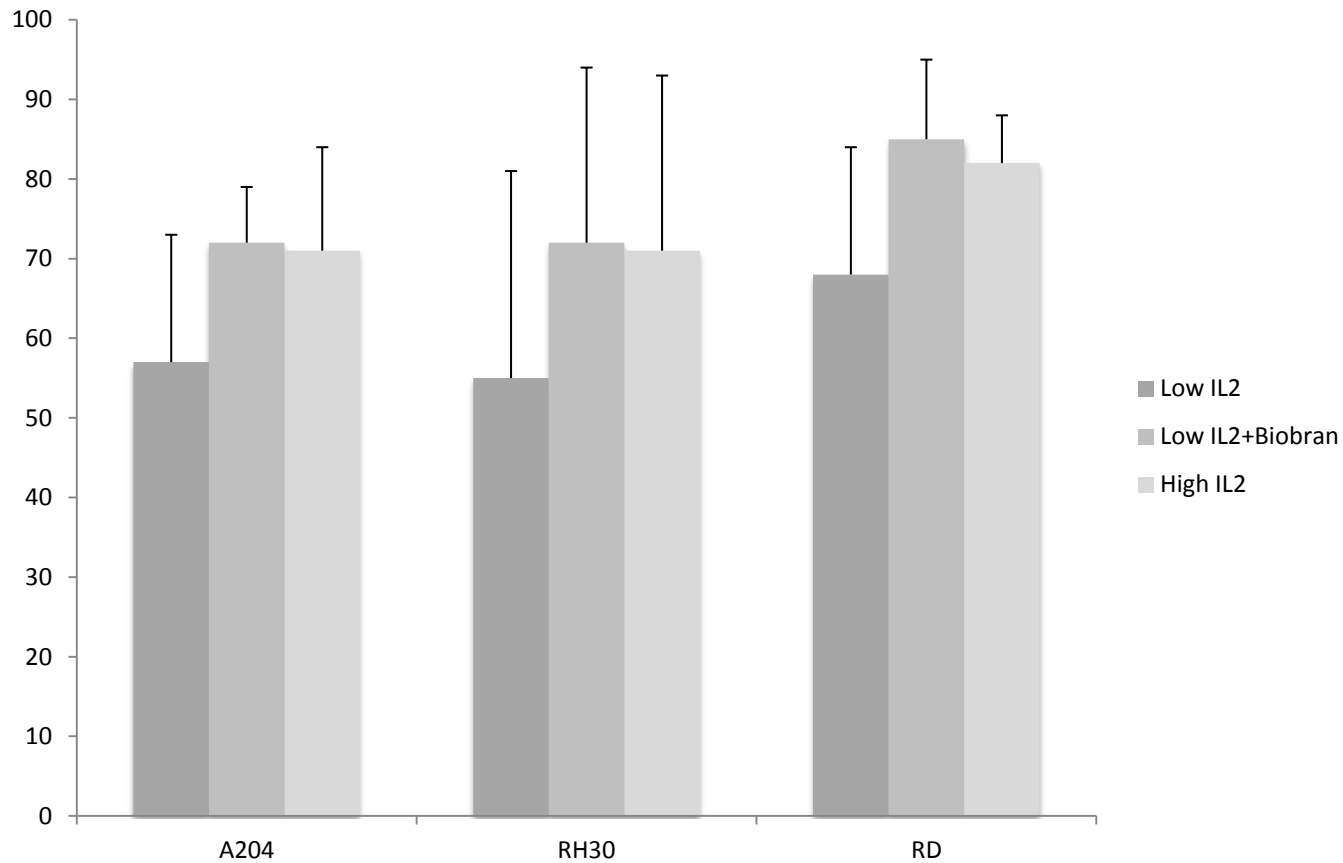
BioBran activated NK cell activity against various tumor cell lines



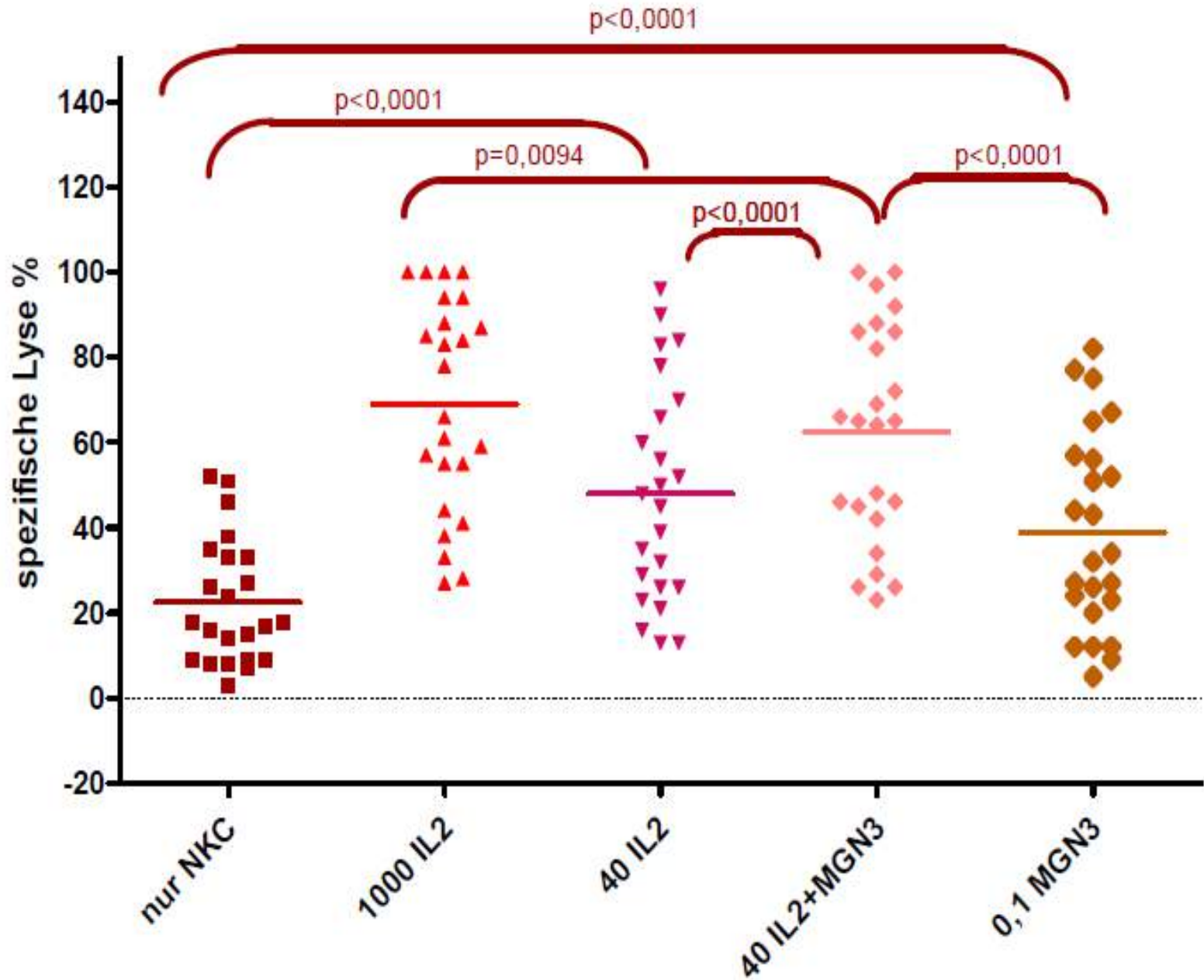
BioBran activated NK cell activity against various tumor cell lines



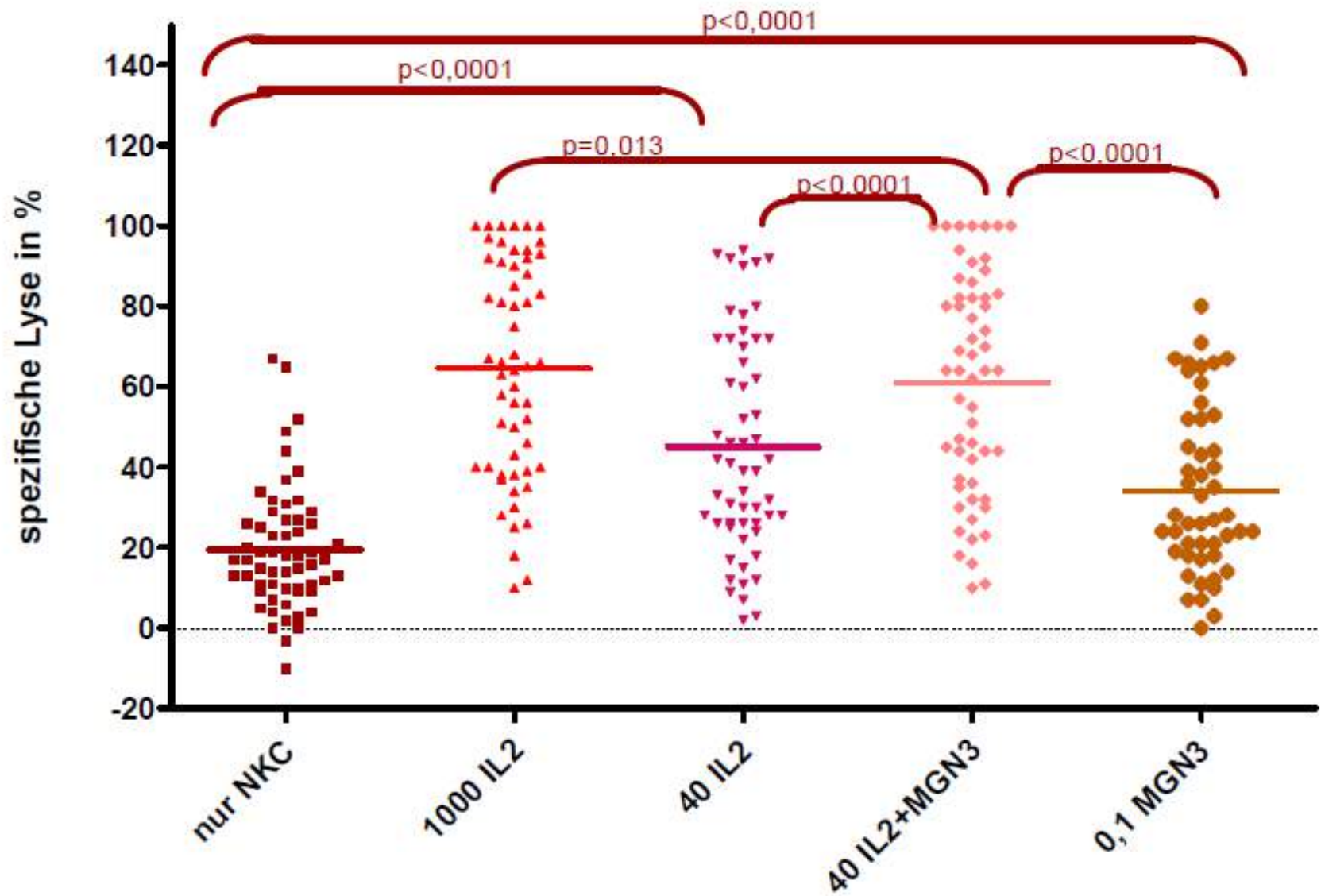
BioBran activated NK cell activity against various tumor cell lines



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



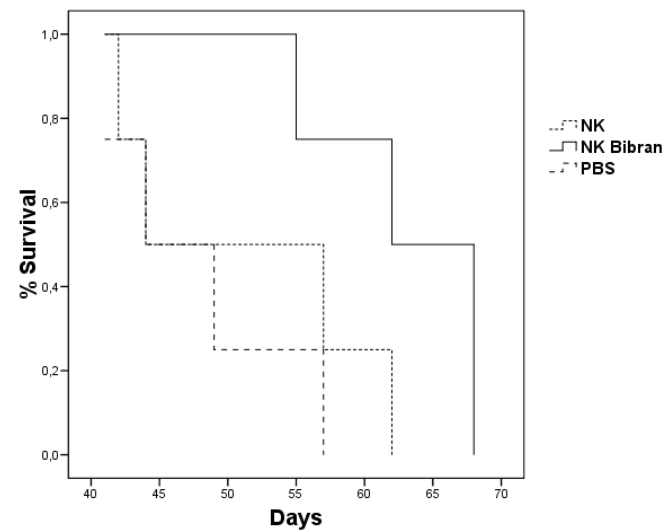
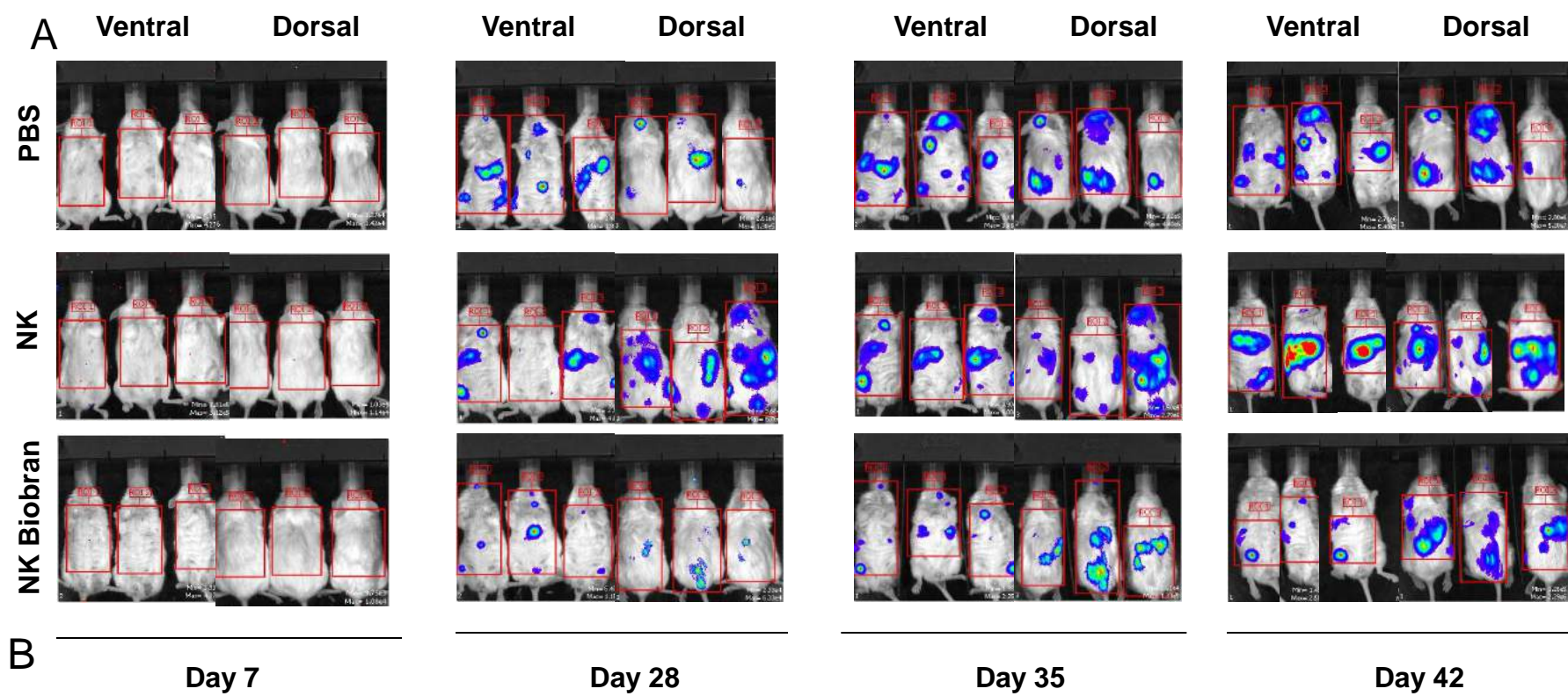
**NK cells
Medium**



**NK cells
Activated overnight
with
BioBran**



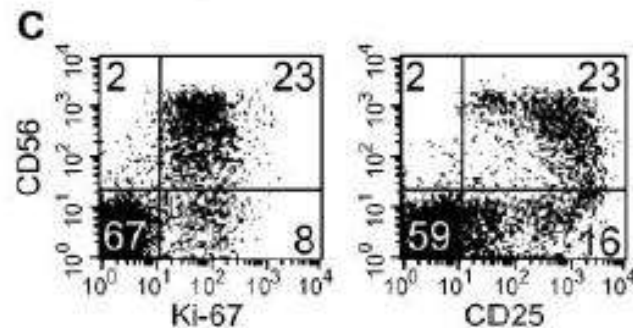
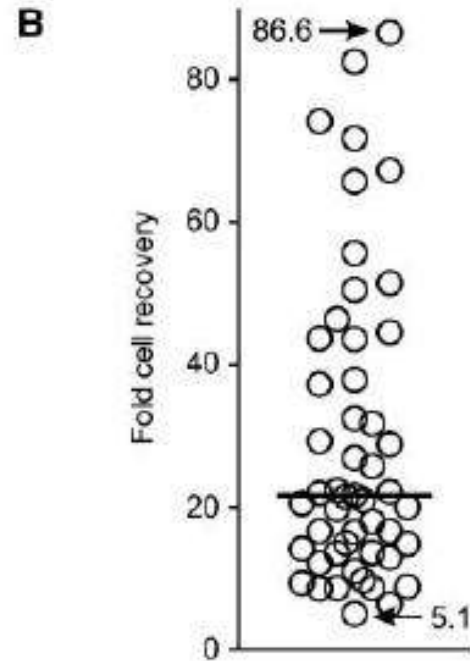
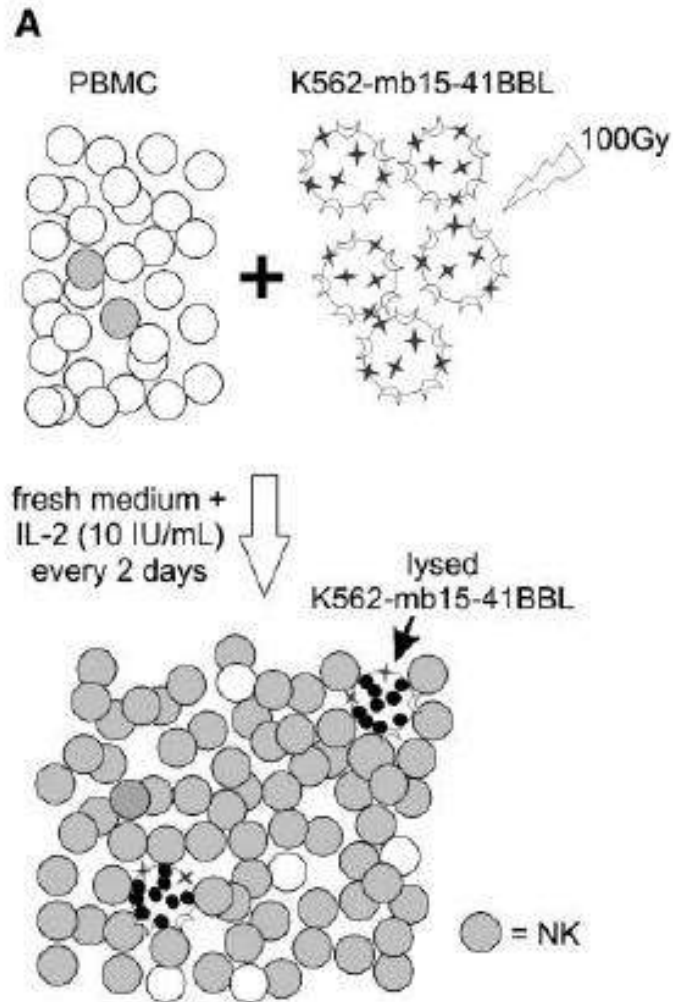
Treatment: twice weekly for 4 weeks



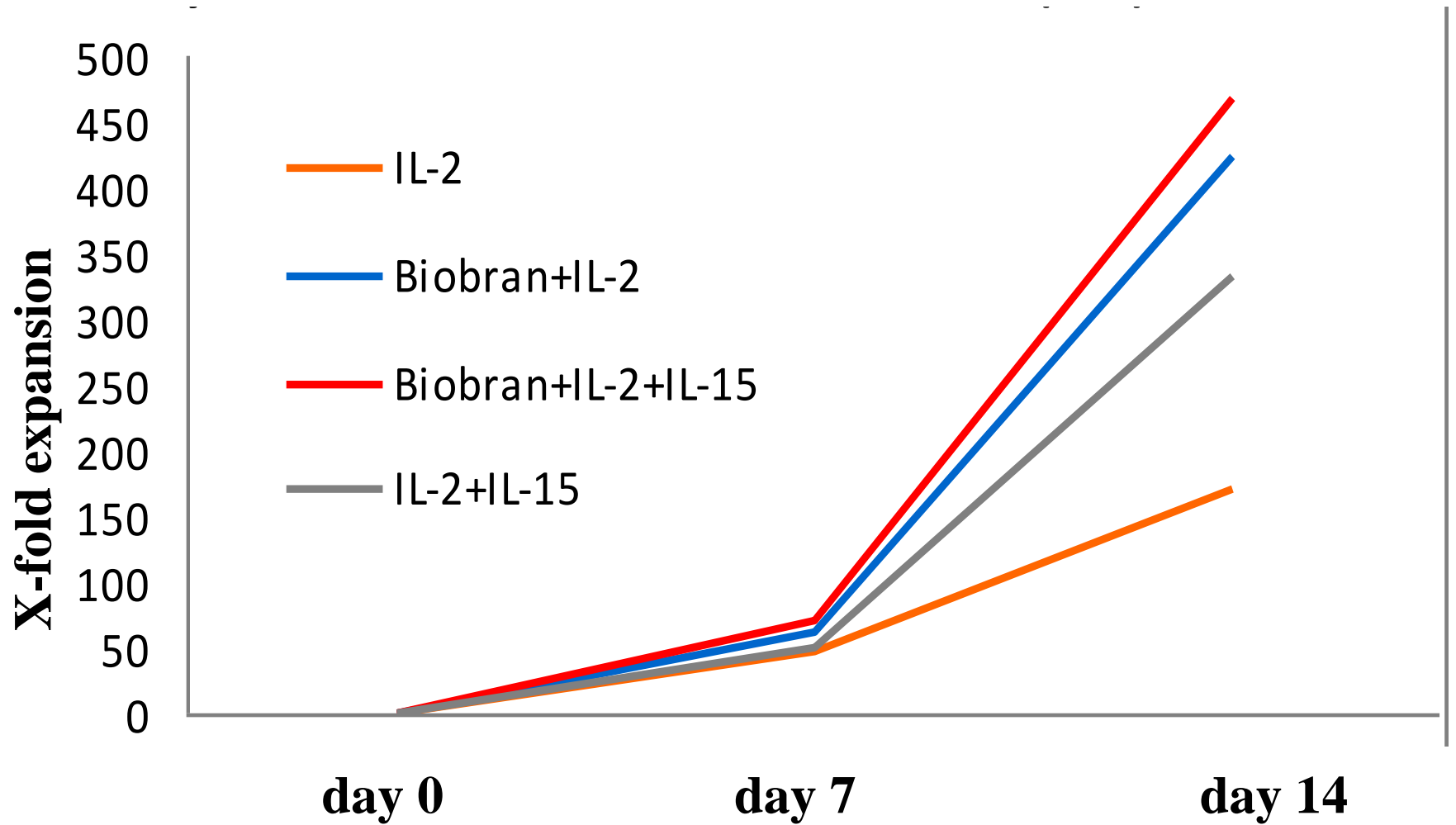
BioBran-stimulated in vitro expansion of NK cells

In vitro expansion von NK-Zellen

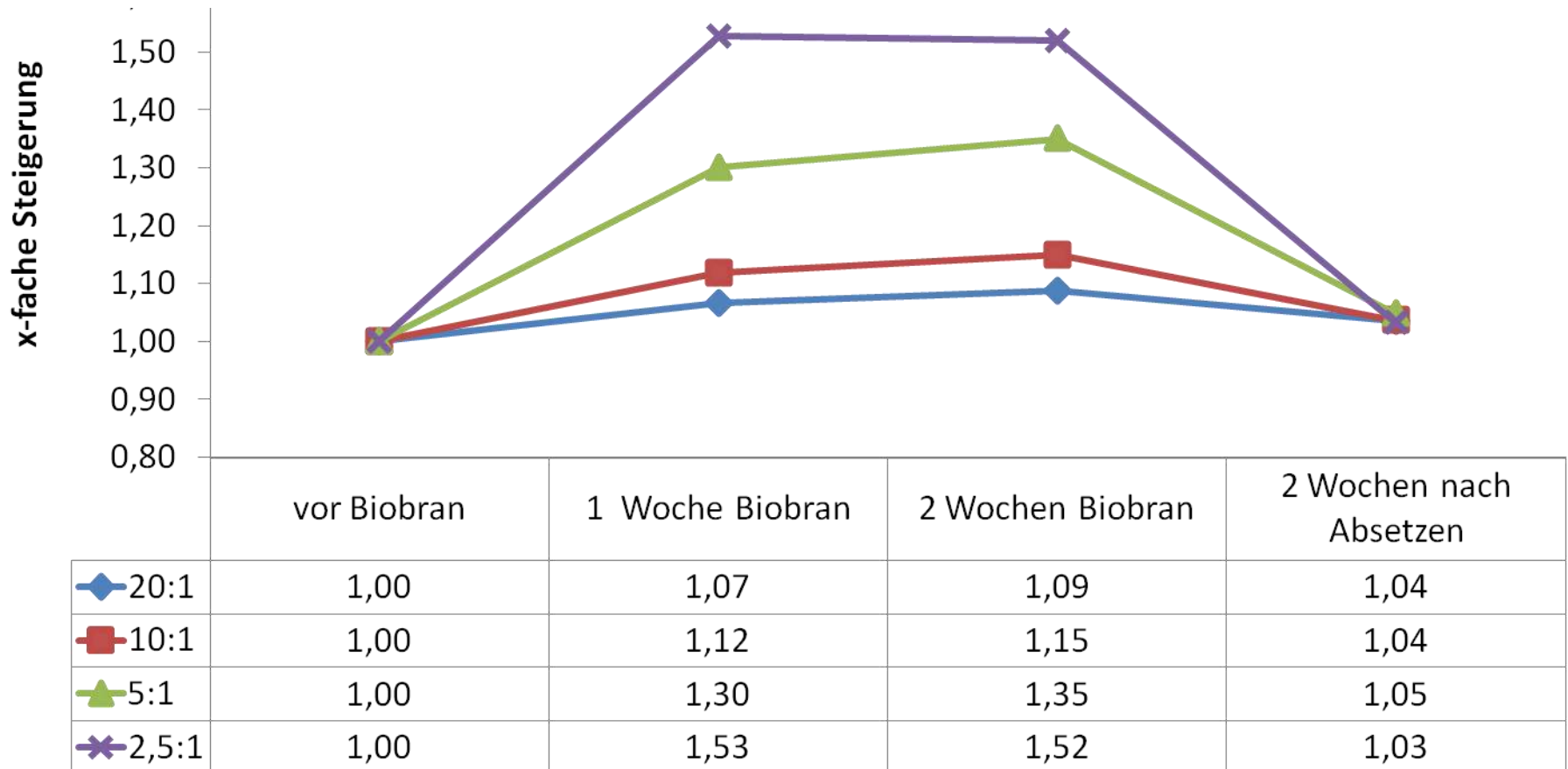
(Dr. Dario Campana, St.Jude Children's Research Hospital, Memphis, USA)



In vitro expansion of NK cells



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



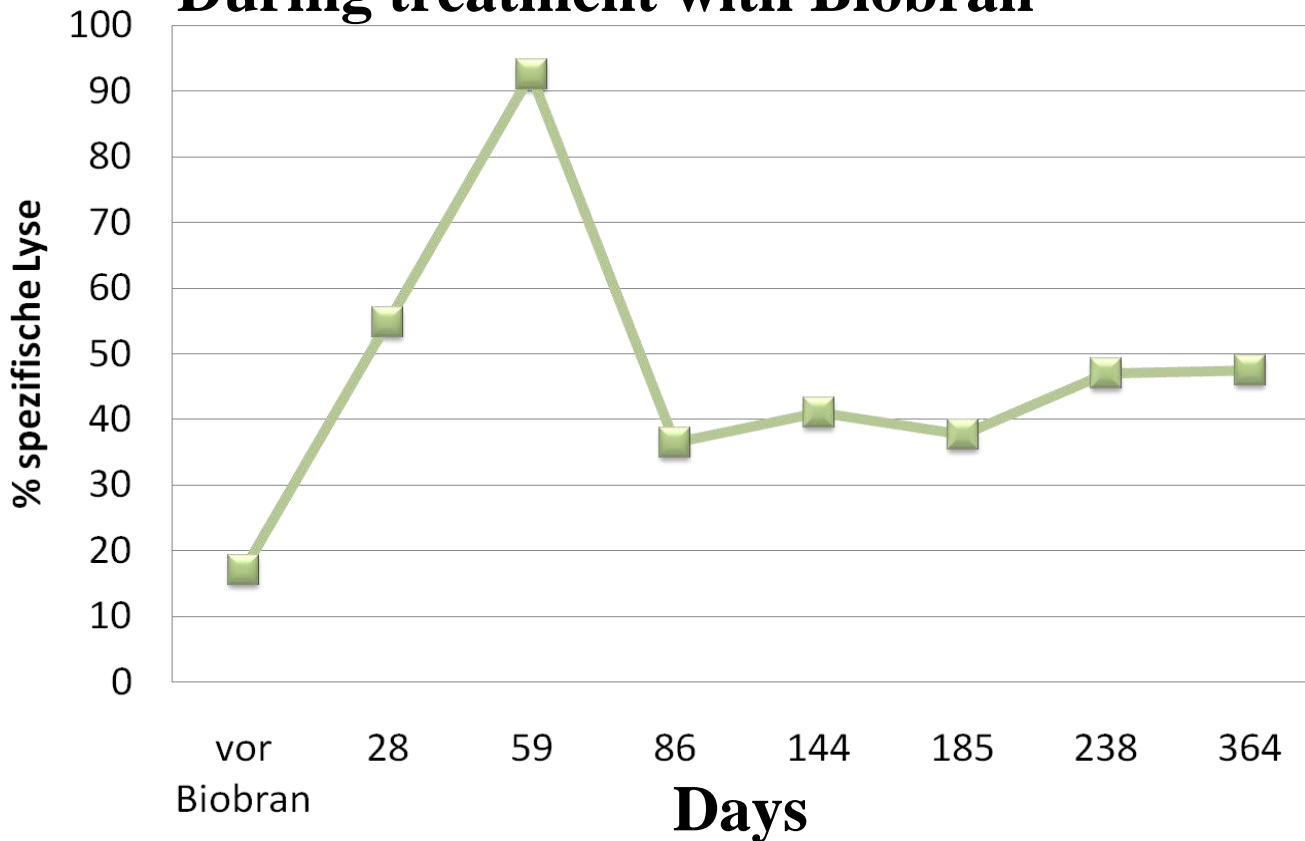
NK-Activity of patients

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

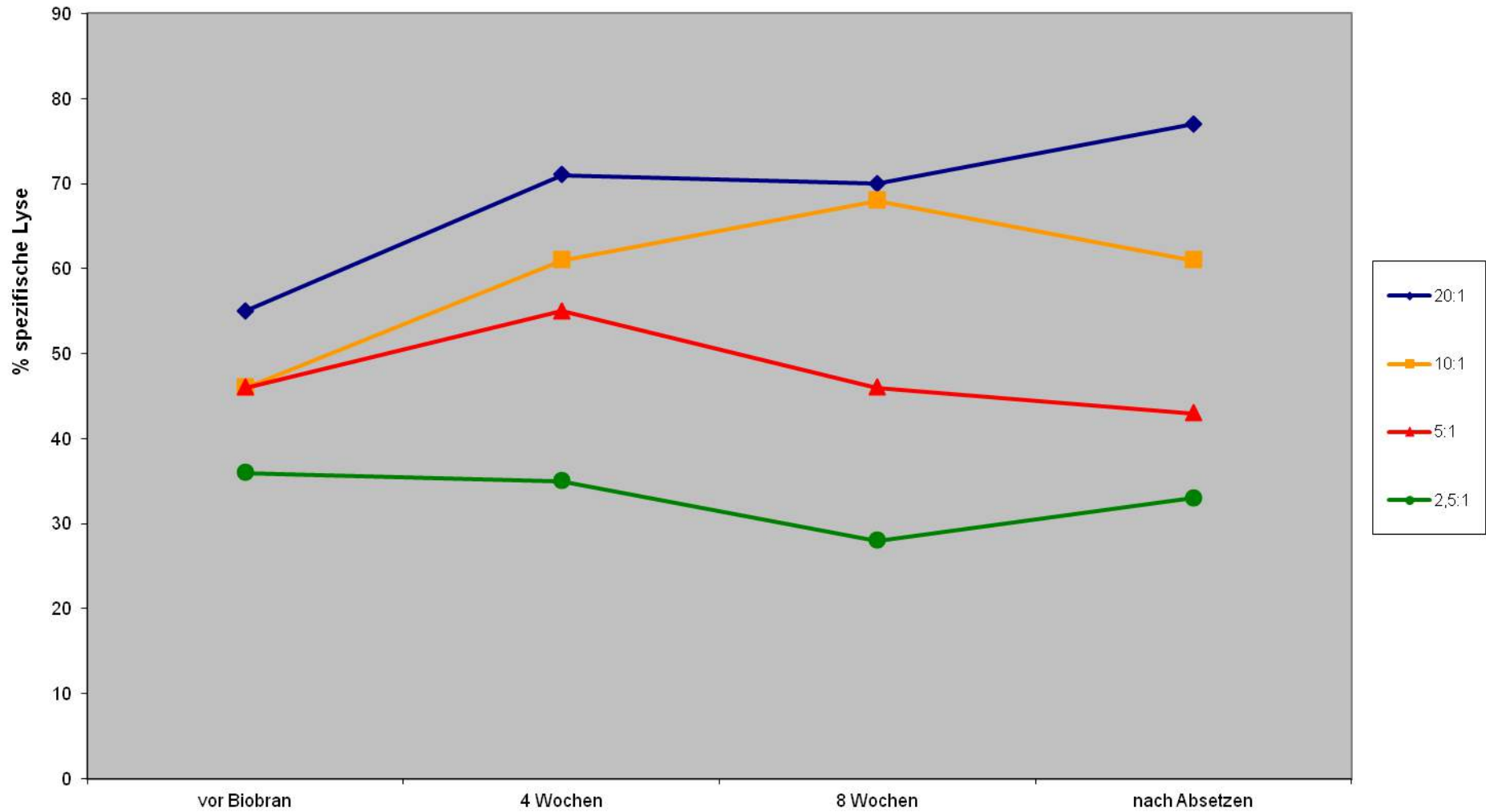
Protokol for pediatric patients with various malignancies

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

NK activity of a patient with neuroblastoma During treatment with Biobran



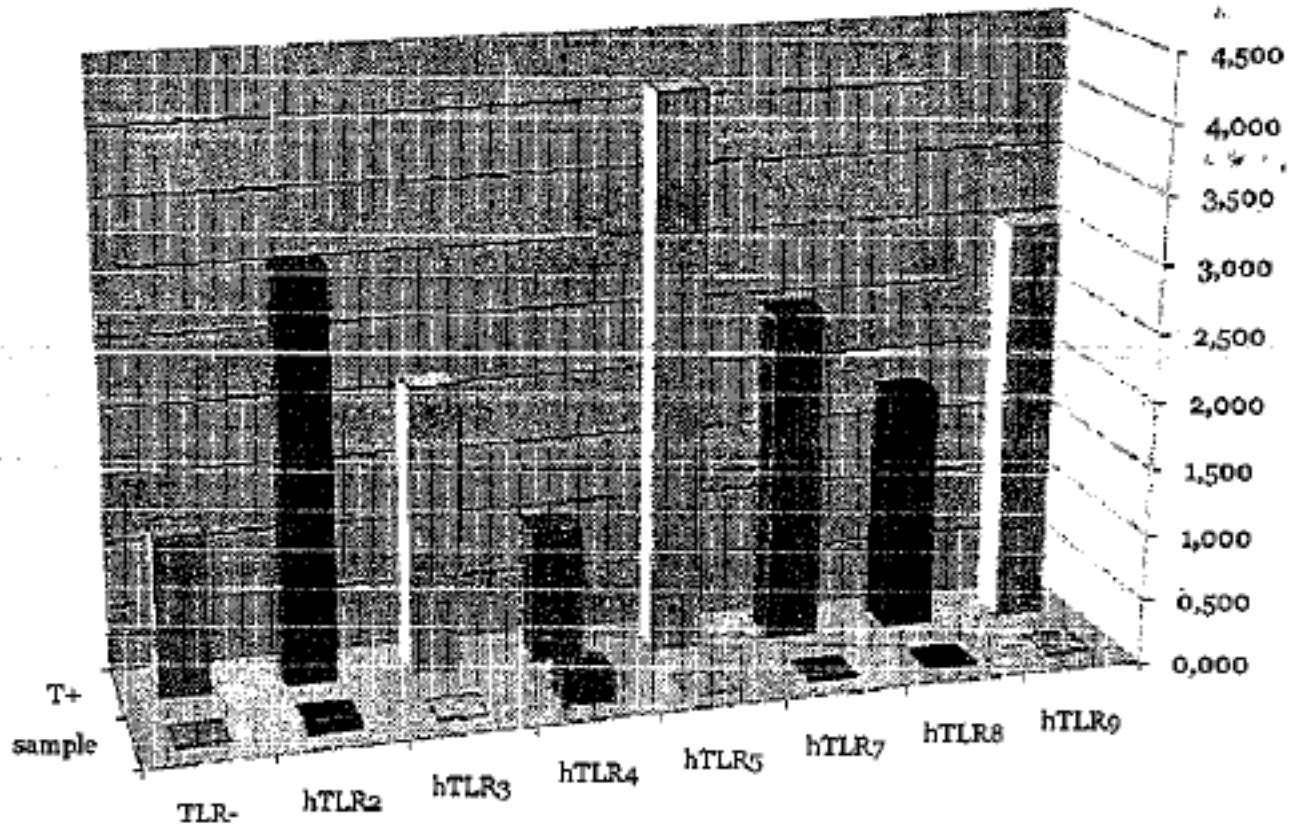
Patient



Patient

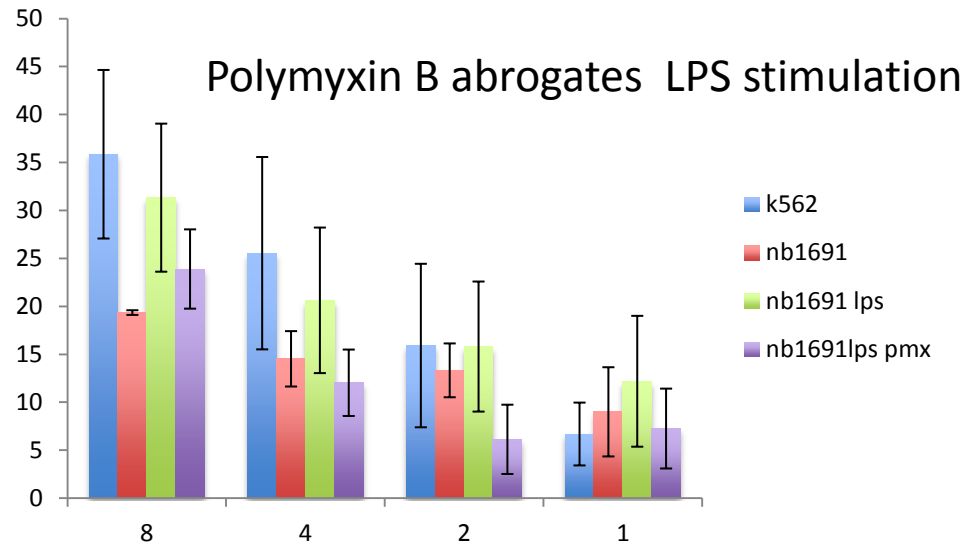


The potential role of Lipopolysaccharide (LPS)

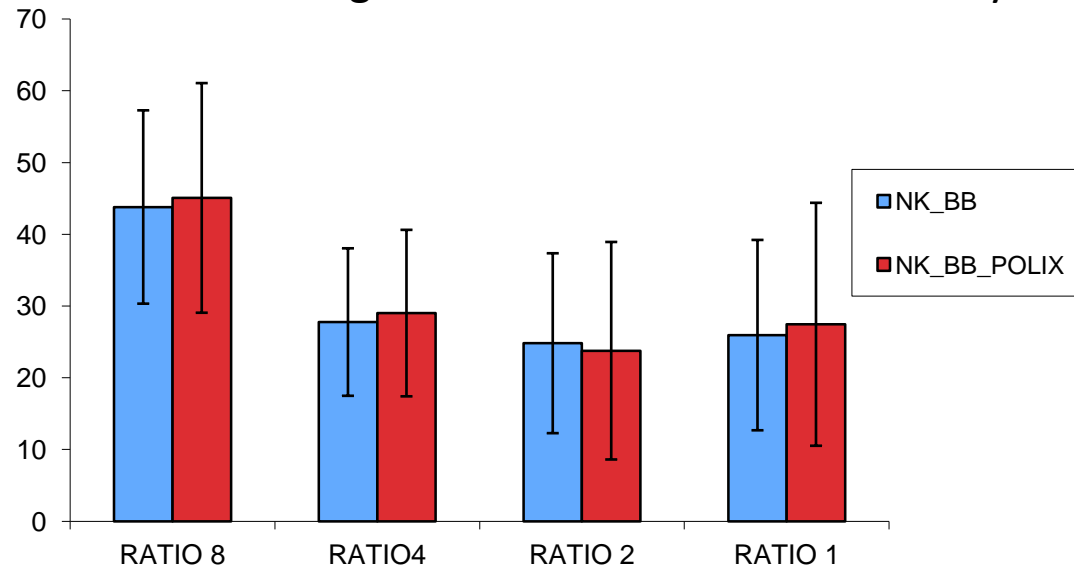


	TLR-	hTLR2	hTLR3	hTLR4	hTLR5	hTLR7	hTLR8	hTLR9
T+	1,233	3,058	2,168	1,012	4,173	2,501	1,818	3,043
sample	0,032	0,080	0,020	0,245	-0,018	0,024	0,020	0,038

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?