

# 1. Internationales Wiener Symposium für Orthomolekulare Medizin

## Immunstimulation und –monitoring beim onkologischen Patienten

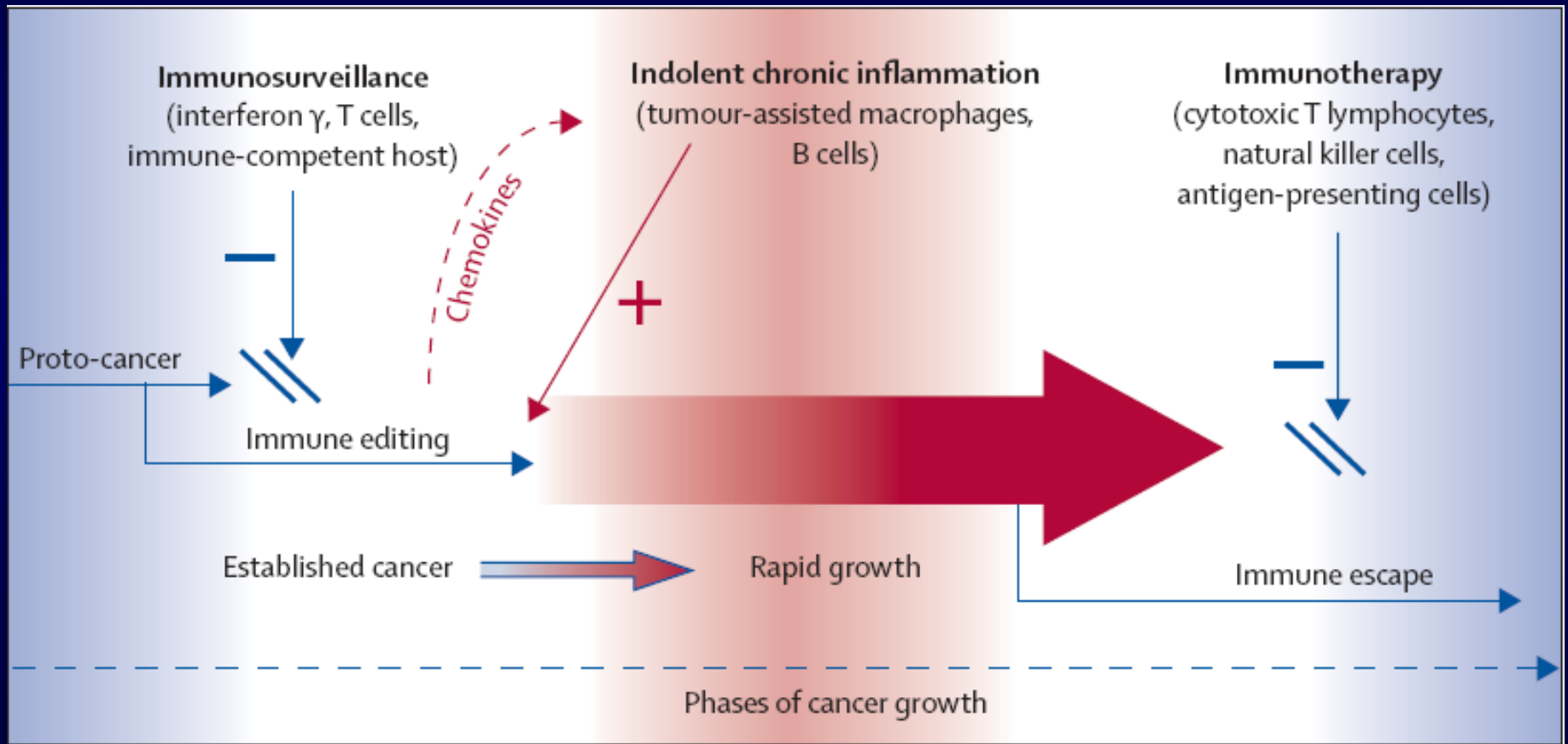
PD Dr. med. WP Bieger – München

*[www.dr-bieger.de](http://www.dr-bieger.de)*

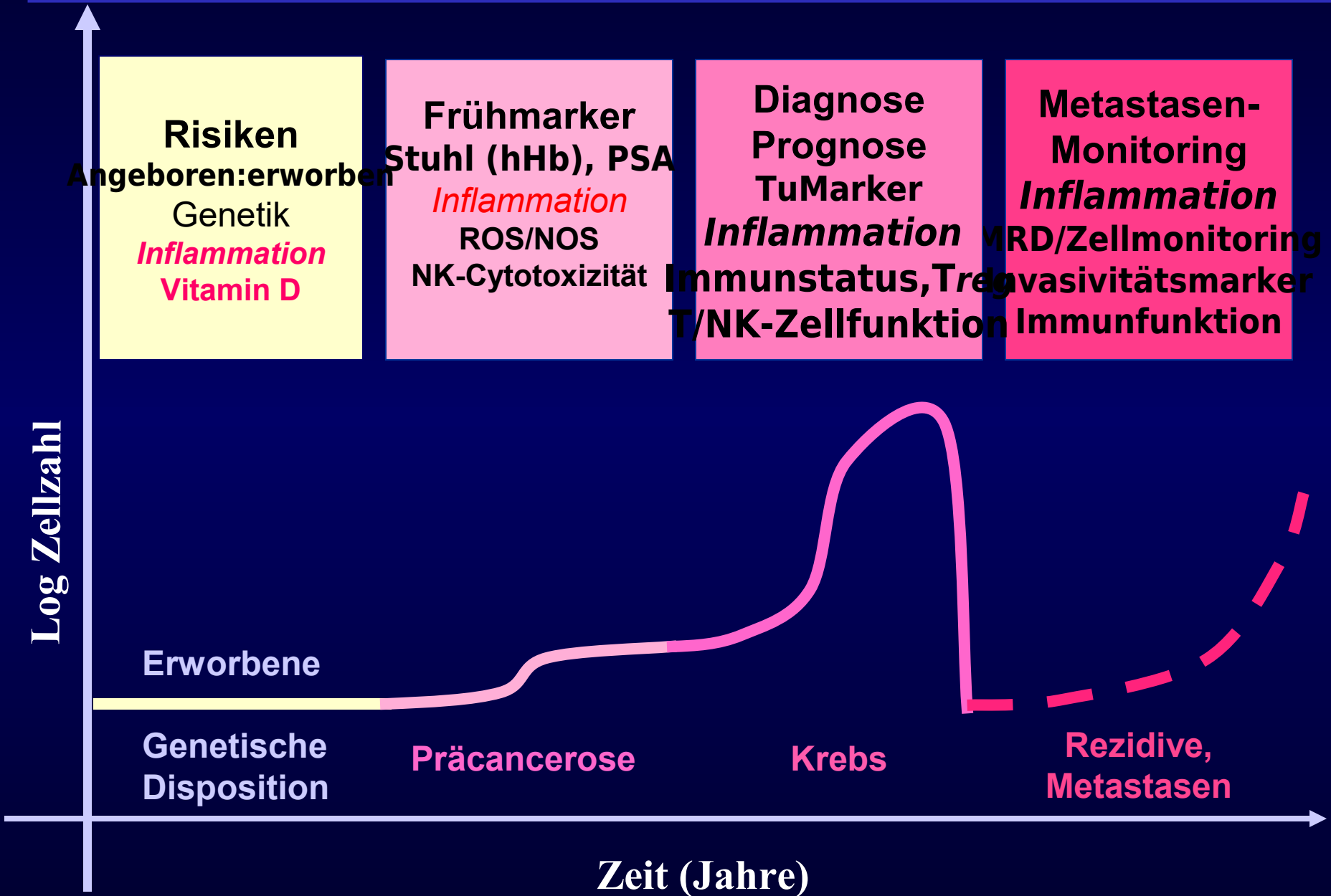
*[praxis@dr-bieger.de](mailto:praxis@dr-bieger.de) – [wbieger@lab4more.de](mailto:wbieger@lab4more.de)*

# Tumour immunity: effector response to tumour and role of the microenvironment

## Postulated interactions between immune and cancer cells at various stages of carcinogenesis and progression



# Stadienabhängige Labordiagnostik von Krebserkrankungen



# TU-Labordiagnostik

## Inflammation

CRPs

TNF alpha

IL-1 $\beta$

IL-6 (sIL6r)

IL-8

IL-12 (IFN $\gamma$ )

IL17

Neopterin

IL-10

TGF beta

MMP-9

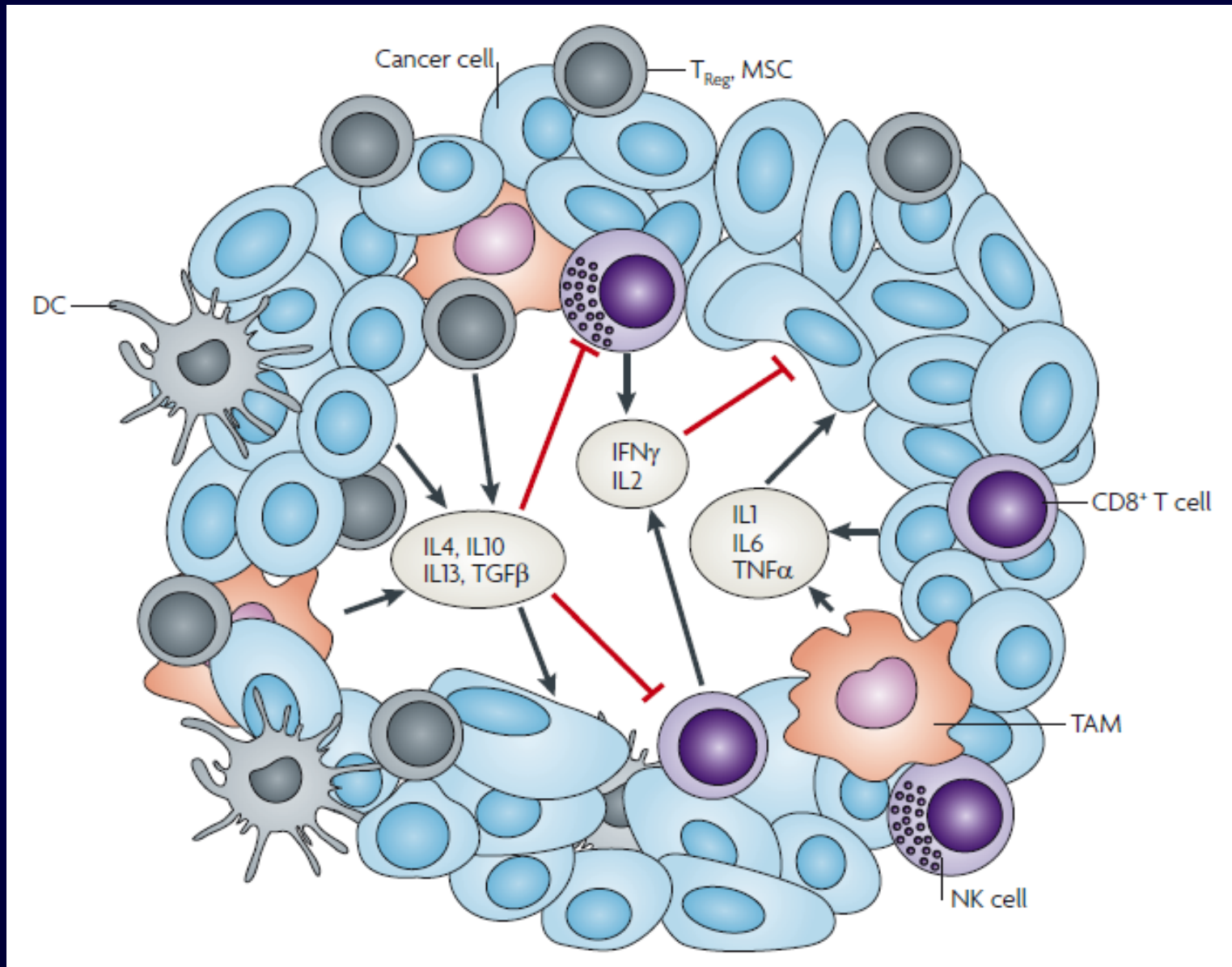
sIL2r

NF-kB

COX2

iNOS

# Role of major cytokines in the tumour microenvironment



# Zytokine im Serum (Metastasierung)



GSH zellulär (CD3)

273



Fimean

> 650



Bitte beachten Sie: geänderter Normbereich ab 05.11.2007!

## VITALSTOFFE



Zink (VB)

623

µg/dl

408 - 760



Selen (VB)

134

µg/l

90 - 150



Selen-Normalverteilung: 50 -120 µg/l; Optimalbereich bei Gesunden: 90 - 150 µg/l, bei Patienten: 120 -250 µg/l

Vitamin C/Ascorbinsäure

3,8

µg/ml

2 - 14



Ubichinon/Q10

1.795

µg/l

900 - 1500



Therapeutisch anzustrebender Bereich: > 2500 µg/l



## Inflammation

CRP sensitiv

0,16

mg/dl

< 0,36



alpha TNF

3,0

pg/ml

< 1



Interleukin 12 (S)

0,0

pg/ml

< 0,2



Interleukin 10 (S)

0,0

pg/ml

< 0,1

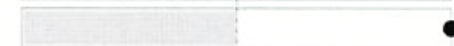


Interleukin 1β (S)

3,0

pg/ml

< 1,0



Interleukin 6 (S)

84,3

pg/ml

< 2



Interleukin 8 (S)

9.444



pg/ml

< 35



sIL2r/lösl.IL2-Rezeptor (S)

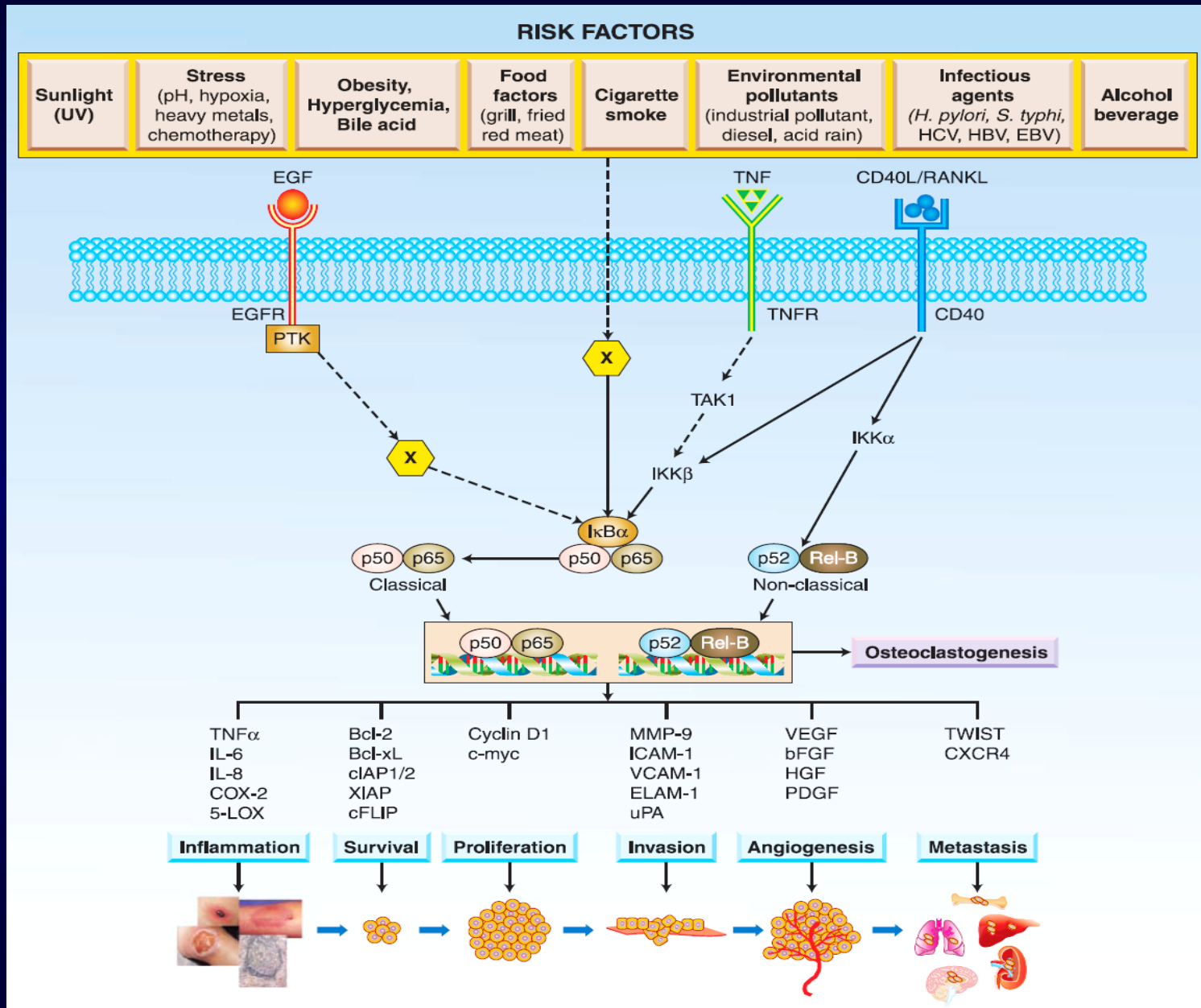
510

U/ml

223 - 710



# Association of NF- $\kappa$ B signaling pathways with tumorigenesis



# Patient MH

Geb. 19.09.1942

830-2549 vom 29.8.2012

## Diagnosen:

Prostata CA

### Inflammation / Zytokine

CRP sensitiv	0,76		mg/dl	< 0,3
<b>ZYTOKINE</b>				
alpha TNF	2.4		pg/ml	< 1
Interleukin 10 (S)	3,1		pg/ml	< 1,5
Interleukin 6 (S)	2,5		pg/ml	< 2
Interleukin 8 (S)	29,9		pg/ml	< 35
TGF-beta (S)	35,3		ng/ml	< 35
COX-2	5,100	●	Qu	< 2,932
NFkB (IkB)	36,71	●	Qu	< 18,368



## Zellulärer Immunstatus

CD4/CD8 T-Zellen, CD4<sup>+</sup>/CD8<sup>+</sup>

Kostimulation (CD3/CD28; CD80/86)

aktivierte T-Zellen: HLADR/CD38/CD25

Chron. T-Zellbelastung (PD-1; CD57, CD28<sup>low</sup>)

regulatorische T-Zellen/Treg (CD25<sup>high</sup>, CD127<sup>low</sup>)

Memory-Zellen (CD45RA/RO, CD62L)

NK-Zellen (CD3<sup>-</sup>, CD56<sup>++</sup>), aktiv. (CD25)

NKT-Zellen (CD3<sup>+</sup>/CD56<sup>++</sup>)

ev. B-Zellen: aktiv. (CD25), unreif (CD5,...), Memory

# Patientin PW

geb, 7.10.1963

830-1748 vom 21.10.2010

3-10

## T-Zellaktivierung

### IMMUNSTATUS (Infekt)

Lymphozyten absolut	2140	/μl	1100 - 4000	
T-Zellen absolut	1797	/μl	920 - 2580	
T-Zellen relativ	84	% CD3	60 - 84	
gamma/delta T-Zellen	126 ←	/μl	< 100	
gamma/delta T %	6	% CD3	< 5	
akt. T-Zellen (HLADR)	158	/μl	< 230	
akt. T (HLADR) %	10	% CD3	< 11	
akt. T-Zellen (CD38) absolut	720	/μl	102 - 554	
akt. T (CD38) relativ	32	% CD3	6 - 28	
akt.T-Zellen (CD25/IL-2)	487 ←	/μl	< 400	
akt.T(CD25) %	31 ←	% CD3	< 22	
CTL (cytotoxische T-Zellen)	13	/μl	10 - 190	
cytotoxische T-Zellen relativ	1	% CD3	1 - 11	
NK-Zellen (absolut)	127	/μl	100 - 600	

# TU-Labordiagnostik



## T-Zellen

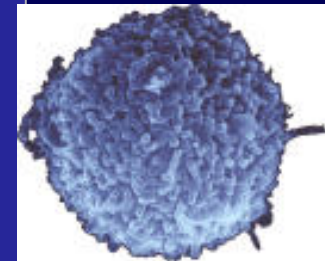
**ITT Zytokin-LTT** :IL-2, IFNg, IL-10  
(TH1/Th2/TH17/Treg)

**T-Select**  $\Rightarrow$  *unter Therapie*

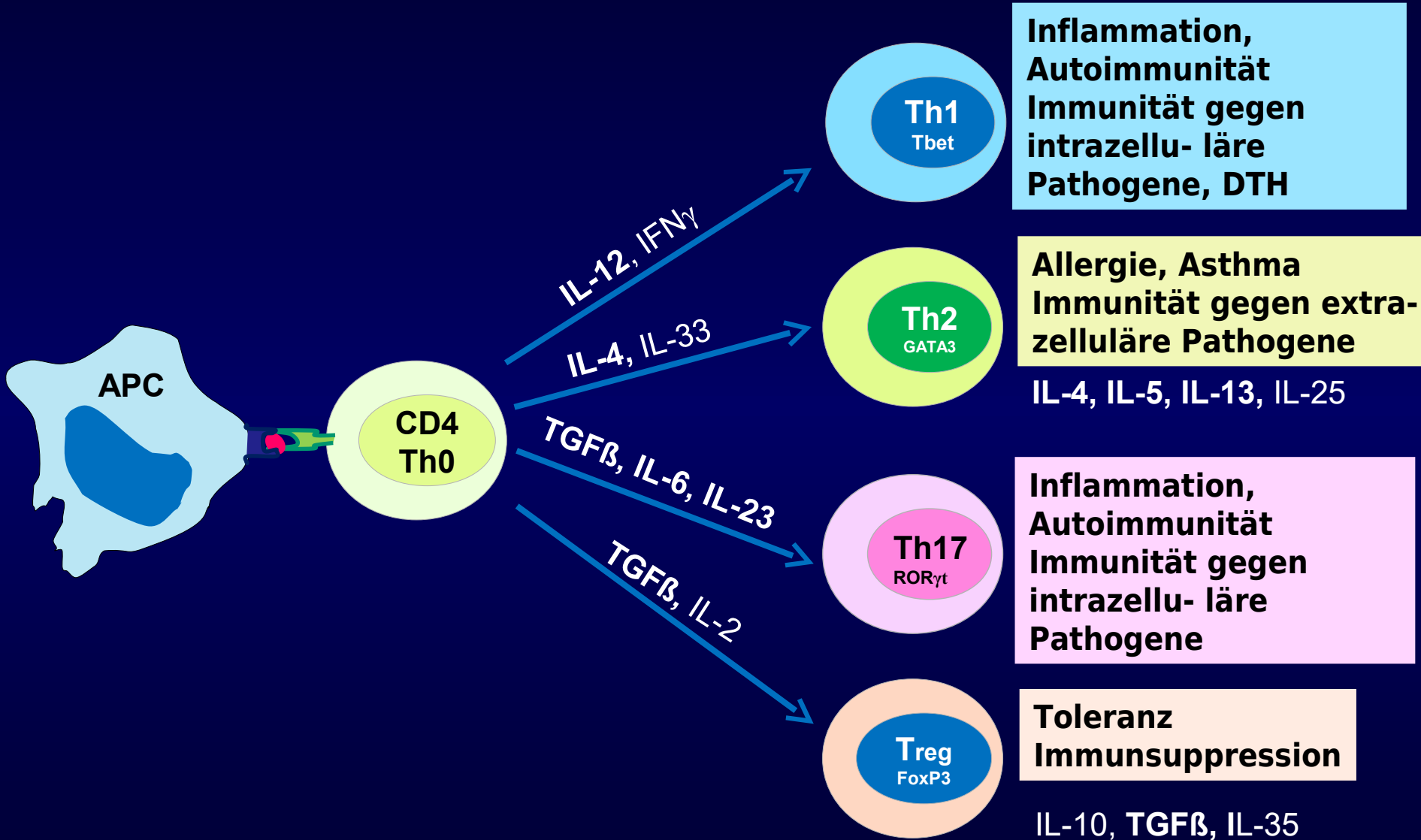
## NK-Zellen

**-NK-Check:** IL-2, CD69 (CD25)

**NK-Select**  $\Rightarrow$  *unter Therapie*



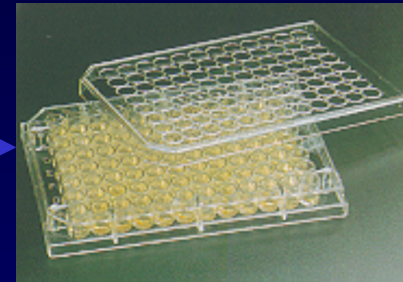
# CD4 -T-Zellreihe



# Zytokin-LTT : ITT



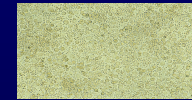
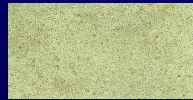
1. Blutabnahme



unstimuliert

Influenza

PWM



IL2

-

+(+)

+++

IFN $\gamma$

-

+

++(+)

IL10

-

-

+(+)

**Patientin Prof KL**

Geb. 09.04.1942

830-2548/23.8.2012; 830-2548/23.8.2012

**Diagnosen:**

Zweitumor Juli 2012

endokrin, undifferenziert

25 J zuvor NasoPharynx-CA

1-5

**CKUPS**

23.08.2012

19.06.2012

**KLEINES BLUTBILD**

	23.08.2012	19.06.2012			
Erythrozyten	4,6	5,0	/pl	4,5 - 5,9	
Hämoglobin	13,2	14,6	g/dl	14 - 17,5	
HBE (MCH)	28,4	29,1	pg	28 - 33	
MCV	87,1	93,2	fl	80 - 98	
Hämatokrit	40,4	46,7	%	36 - 48	
MCHC	32,7	31,3	g/dl	33 - 36	
RDW (Ery)	14,2	15,6	%	11 - 16	
Thrombozyten	337	251	TSND/μl	140 - 400	
Leukozyten	9,6	4,7	/nl	4 - 10	
MPV	9,8	10,3	fl	7,8 - 11,5	
Neutrophile	73,0	54,1	%	30 - 85	
Lymphozyten	13,2	30,5	%	20 - 52	
Monozyten	12,7	12,9	%	2 - 12	
Granulozytenzahl abs	6,97	2,55	/nl	2,4 - 7,4	
Lymphozytenzahl abs	1,26	1,44	/nl	1,1 - 4,0	
Monozytenzahl abs	1,21	0,61	/nl	0,14 - 0,8	

**Patientin Prof KL**  
 Geb. 09.04.1942  
 830-2548/23.8.2012; 830-2548/23.8.2012

**Diagnosen:**  
 ZweitTumor Juli 2012  
 endokrin, undifferenziert  
 25 J zuvor NasoPharynx-CA

23.08.2012    19.06.2012

**IMMUNSTATUS (TU)**

	<u>23.08.2012</u>	<u>19.06.2012</u>			
Lymphozyten absolut	1260	1440	/µl	1100 - 4000	
Lymphozyten relativ	13	31	%	20 - 52	
Granulozyten	6970	2550	/µl	2400 - 7400	
Granulo %	73	54	%	42 - 75	
T-Zellen absolut	498	662	/µl	920 - 2580	
T-Zellen relativ	40	46	% Lympho	60 - 84	
CD4-Helferzellen absolut	401	484	/µl	550 - 1660	
CD4-Helferzellen relativ	32	34	%CD3	32 - 60	
CD8-Zellen: absolute	73	125	/µl	280 - 930	
CD8-Zellen: relativ	6	9	%CD3	13 - 40	
CD4/CD8 RATIO	5,46	3,88		1,0 - 2,8	
CD3+/CD4+/CD8+	0	0	% Lymph	< 5	



T-Zell-Aktivierung	<u>23.08.2012</u>	<u>19.06.2012</u>			
akt. T-Zellen (CD38) absolut	51	69	/µl	102 - 554	
akt. T-Zellen (HLADR)	31	29	/µl	< 230	
akt. T (HLADR) %	6	5	% CD3	< 11	
akt. T (CD38) relativ	10	11	% CD3	6 - 28	
<b>cytotoxische Zellen</b>					
CD86 auf APC relativ	2		% APC	5 - 25	
cytotoxische T-Zellen relativ	3	4	% CD3	1 - 11	
<b>Kostimulatorische Signale</b>					
CD4/costim/CD28 relativ	98		% CD4	98 - 100	
CD8/costim/CD28 relativ	66		% CD8	> 50	

ZYTOKINE		...			
Interleukin 10 (S)	2,3		pg/ml	< 1,5	
Interferon-gamma (S)	32,5	←	pg/ml	< 1,0	
TGF-beta (S)	23,1	23,1	ng/ml	< 35	
MCP-1	154		pg/ml	< 50	



## ITT-Immuntoleranztest (TH1) 23.08.2012 19.06.2012

### ITT Basal

	23.08.2012	19.06.2012	basal		
IL-2 Basal (TH1)	0,0	0,0	pg/ml	< 1	
IL-10 Basal (TH3)	0,4	0,0	pg/ml	< 3	
Interferon Basal (TH1)	0,0	0,0	pg/ml	< 1	

### Kontrolle I

	PWM	PWM			
IL-2m Kultur 1	749,8	1434,0	pg/ml	> 100	
IL-10m Kultur 1	730,3	316,4	pg/ml	> 50	
IFN-gamma Kultur 1	5789,0	4126,1	pg/ml	> 200	

### Antigen 2

	Influenza	Influenza			
IL-2m Kultur 2	2,1	31,2	pg/ml	> 15	
IL-10m Kultur 2	3,3	2,4	pg/ml	< 10	
INF-gamma Kultur 2	0,0	45,0	pg/ml	> 10	

### NK-Zellfunktion

NK Zellen cytotoxisch	98	% NK	85 - 95	
NK Zellen regul.	2	%	5 - 15	

### NK-Check®







	23.08.2012	19.06.2012	...		
NK/Ctx: basal	40	17	% K562	> 20	
NK/Ctx: IL-2 stim	40	35	% K562	> 35	
NK-Zellen CD69 basal	1	11	% NK		
NK-Zellen CD 69 IL2	27	41	% NK		

## Tumorscreening

CA 125 (Roche)	314,0		U / ml	< 35	
CYFRA 21-1	5,5		ng/ml	< 3,3	
Calcitonin	<2,0		pg/ml	< 20	
CA15-3 (Roche)	91,1		U/ml	< 25	
<b>Tumorinvasivität</b>					
VEGF	121		pg/ml	< 100	
FGF	23		pg/ml	< 10	
uPAR	4,6		ng/ml	< 3	
NSE(Roche)	124,0 ←		µg/l	< 18,3	
Septin 9	negativ			negativ	
CEA (S/Siemens)	2,0	2,0	ng/ml	< 4,7	
PSA	5,20	5,13	ng/ml	< 4	
PSA frei	0,478	0,510	ng/ml	< 0,4	
PSA - Quotient	0,09	0,10	Quotient	> 0,23	
cPSA komplexiert	3,40	4,00	ng/ml	< 2,5	
p53-AK (Dianova)	<60	<60	U/ml	< 60	
ChromograninA (CgA)	112,0 ←		ng/ml	< 100	

# Aktivierete T-Zellen

## ITT-Immunkfunktion

Basal	Basal			
IL-2 Basal (TH1)	121,6	pg/ml	< 1	
IL-10 Basal (TH3)	368,4	pg/ml	< 3	
INF-gamma Basal (TH1)	504,4	pg/ml	< 1	
<b>Pos. Kontrolle 1</b>	<b>PWM</b>			
IL-2 Pos. Kontrolle (TH1)	583,8	pg/ml	> 100	
IL-10 Pos. Kontrolle (TH3)	800,2	pg/ml	> 50	
INF-gamma Pos. Kontrolle (TH1)	959,6	pg/ml	> 200	
<b>Antigen 1</b>	<b>Influenza</b>			
IL-2 (TH1)	169,3	pg/ml	> 15	
IL-10 (TH3)	461,6	pg/ml	< 10	
INF-gamma (TH1)	547,2	pg/ml	> 10	

# TH2 Dominanz + Inflammation

## Patientin T1

Geb.27.06.1965

740-0541 vom 22.6.2011

## Diagnosen:

Mamma-CA, metastasierend

ITT Basal	basal			
IL-2 Basal (TH1)	5,7	pg/ml	< 1	
IL-10 Basal (TH3)	6,0	pg/ml	< 3	
Interferon Basal (TH1)	19,4	pg/ml	< 1	
Pos. Control 1	PWM			
IL-2 (TH1)	234,1	pg/ml	> 100	
IL-10 (TH3)	316,0	pg/ml	> 50	
INF-g (TH1)	289,2	pg/ml	> 200	
Antigen 1	Influenza			
IL-2 (TH1)	15,6	pg/ml	> 15	
IL-10 (TH3)	110,4	pg/ml	< 10	
INF-g (TH1)	8,9	pg/ml	> 10	



# T-Zell Anergie

## T-Zellfunktion

### ITT-Immunfunktion

Basal		...			
IL-2 Basal	0,0	pg/ml	< 1		
IL-10 Basal	0,0	pg/ml	< 3		
INF-gamma Basal	0,0	pg/ml	< 1		
Pos. Kontrolle 1		PWM			
IL-2 Pos. Kontrolle	268	pg/ml	> 100		
IL-10 Pos. Kontrolle	18,8	pg/ml	> 50		
INF-gamma Pos. Kontrolle	91,5	pg/ml	> 200		
Antigen 1		Influenza			
IL-2	0,0	pg/ml	> 15		
IL-10	0,0	pg/ml	< 20		
INF-gamma	0,0	pg/ml	> 5		



**70 jähriger Patient, bei dem vor 10 Jahren ein Prostata-CA diagnostiziert wurde. Er lehnte konventionelle Therapien ab (OP, Chemotherapie) und befolge in den anschließenden Jahren ein eigenes Regime mit *Zink, Carnitin, Vitamin E, Selen, Granatapfel, Vitamin D, Quercetin, Curcumin, Sitosterol.***

**Anfang 2012 begann PSA zu steigen – ohne jede klinische Symptomatik. Er intensivierte die Supplementierung und begann vor 8 Wochen die intensivierte Immuntherapie mit *GcMAF.***

**Patient MH**

Geb. 19.09.1942

830-2549 vom 29.8.2012

# Immunistatus

3-8

**Diagnosen:**

Prostata CA

## IMMUNSTATUS Klein

Lymphozyten absolut	1060	●	/μl	1100 - 4000
Lymphozyten relativ	25		%	20 - 52
Monozyten	500		/μl	140 - 800
Mono %	12		%	2 - 14
Granulozyten	2390		/μl	2400 - 7400
Granulo %	56		%	42 - 75
T-Zellen absolut	780		/μl	920 - 2580
T-Zellen relativ	74		% Lympho	60 - 84
gamma/delta T-Zellen	37		/μl	< 100
gamma/delta T %	5		% CD3	< 5
CD4-Helferzellen absolut	486		/μl	550 - 1660
CD4-Helferzellen relativ	46		%CD3	32 - 60
CD8-Zellen: absolute	264		/μl	280 - 930
CD8-Zellen: relativ	25		%CD3	13 - 40
CD4/CD8 RATIO	1,84			1,0 - 2,8
CD3+/CD4+/CD8+	1		% Lymph	< 5
CTL (cytotoxische T-Zellen)	24		/μl	10 - 190
cytotoxische T-Zellen relativ	3		% CD3	1 - 11

**Patient MH**  
Geb. 19.09.1942  
830-2549 vom 29.8.2012

# „Tumormarker“

**Diagnosen:**  
Prostata CA

Tumorscreening			
PSA (Siemens)	33,800	ng/ml	< 4,5
PSA frei (Siemens)	3,090	ng/ml	< 0,4
PSA - Quotient	0,09	Quotient	> 0,23
cPSA komplexiert	24,00	ng/ml	< 2,8
uPAR	3,9	ng/ml	< 3
Nagalase-Aktivität	2,14	E	0,2 - 0,95
GSH zellulär (CD3)	552	Fimean	> 650



**Patient MH**

Geb. 19.09.1942

830-2549 vom 29.8.2012

**Diagnosen:**

Prostata CA

4-8

## Inflammation

### Inflammation / Zytokine

CRP sensitiv	0,76		mg/dl	< 0,3
<b>ZYTOKINE</b>				
alpha TNF	2.4		pg/ml	< 1
Interleukin 10 (S)	3,1		pg/ml	< 1,5
Interleukin 6 (S)	2,5		pg/ml	< 2
Interleukin 8 (S)	29,9		pg/ml	< 35
TGF-beta (S)	35,3	●	ng/ml	< 35
COX-2	5,100		Qu	< 2,932
NFkB (IkB)	36,71	●	Qu	< 18,368

**T-Zell-Aktivierung**

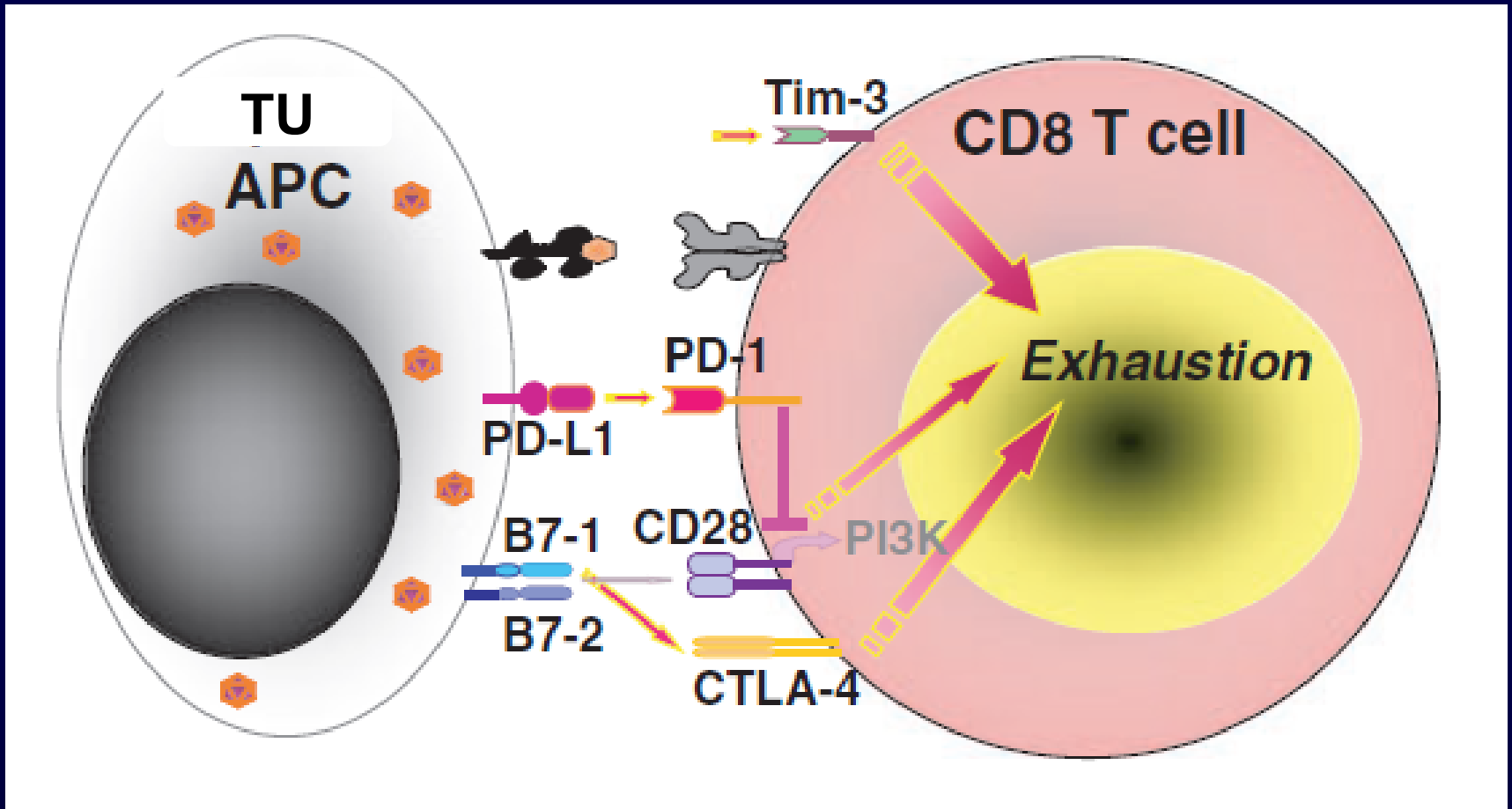
akt. T-Zellen (HLADR)	105	/μl	< 230
akt. T (HLADR) %	14 ●	% CD3	< 11
akt. T-Zellen (CD38) absolut	99	/μl	102 - 554
akt. T (CD38) relativ	13	% CD3	6 - 28
akt. T (CD25) %	5	% CD3	< 22
akt. T-Zellen (CD25/IL-2)	43	/μl	< 400
CTL (cytotoxische T-Zellen)	24	/μl	10 - 190

**„T-Zell-Erschöpfung“**

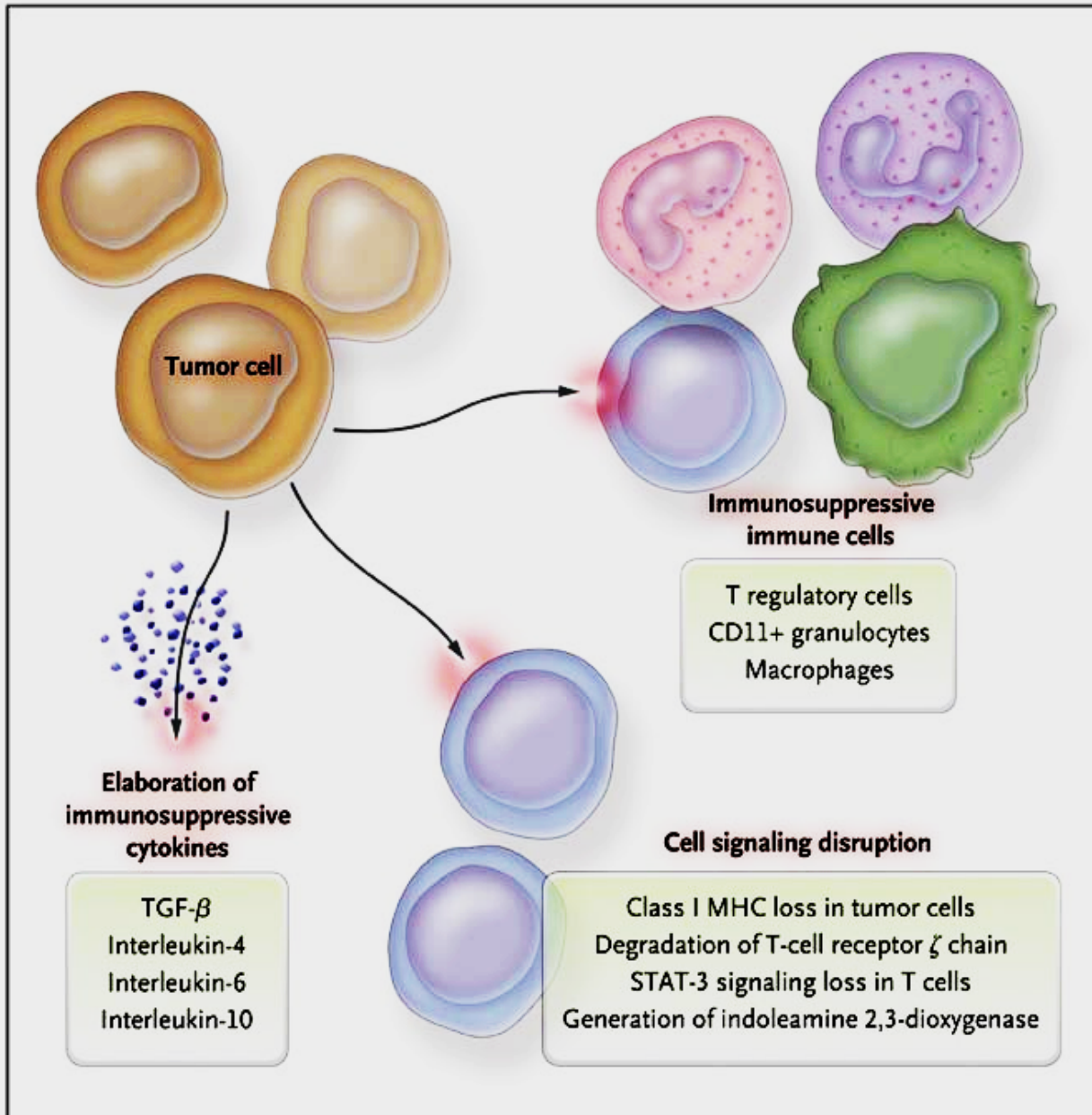
T-Zellen PD1:relativ	3,8 ●	% CD3	< 2
CD8-Zellen PD1:relativ	1,9	% CD8	< 1
CD8-Zellen senesc (CD57)	108	/μl	10 - 387
senCD8-Zellen (CD57) %	41	% T-Zellen	1 - 15

# T cell Exhaustion

Signalling through multiple cell surface inhibitory receptors like PD-1, CTLA-4 and Tim-3 has been associated with the T-cell dysfunction in Virus infection or Tumor.



# Tumor-Derived Immunosuppression



Weiner L: Cancer Immunotherapy  
— The Endgame Begins.  
N Engl J Med 2008;358:2664-2665

**Patient MH**

Geb. 19.09.1942

830-2549 vom 29.8.2012

5-8

# T-Zellfunktion

**Diagnosen:**

Prostata CA

## ITT-Immundefizienz

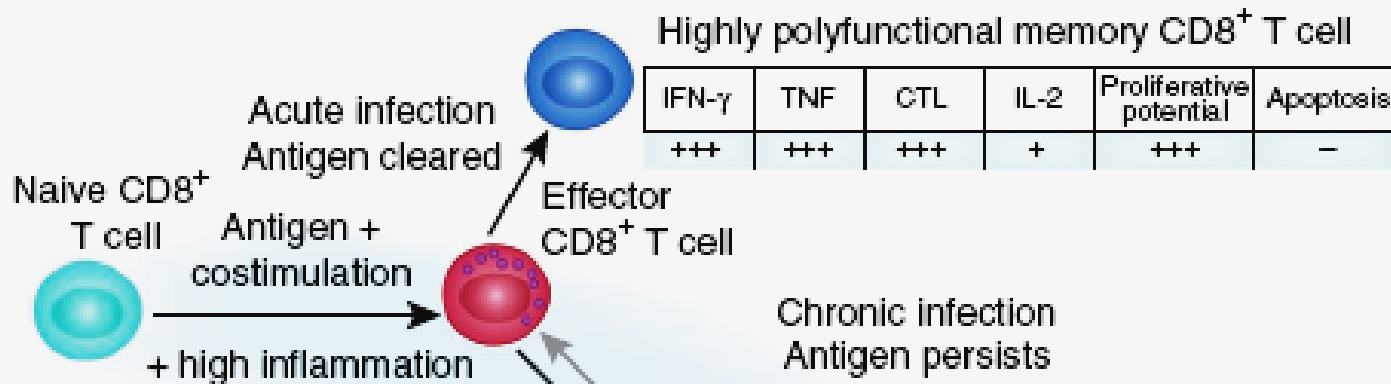
<b>Basal</b>	<b>basal</b>		
IL-2 Basal (TH1)	0,0	pg/ml	< 1
IL-10 Basal (TH3)	0,4	pg/ml	< 3
INF-gamma Basal (TH1)	0,0	pg/ml	< 1
<b>Pos. Kontrolle 1</b>	<b>PWM</b>		
IL-2 Pos. Kontrolle (TH1)	257,8	pg/ml	> 100
IL-10 Pos. Kontrolle (TH3)	503,5	pg/ml	> 50
INF-gamma Pos. Kontrolle (TH1)	1162,4	pg/ml	> 200
<b>Antigen 1</b>	<b>Influenza</b>		
IL-2 (TH1)	36,2	pg/ml	> 15
IL-10 (TH3)	1,7	pg/ml	< 10
INF-gamma (TH1)	4,8 ●	pg/ml	> 10
<b>Antigen 2</b>	<b>OKT3</b>		
IL-2 (TH1)	42,1	pg/ml	> 5
IL-10 (TH3)	88,8	pg/ml	< 10
IFN-gamma (TH1)	14,7 ●	pg/ml	> 20

# T cell exhaustion.

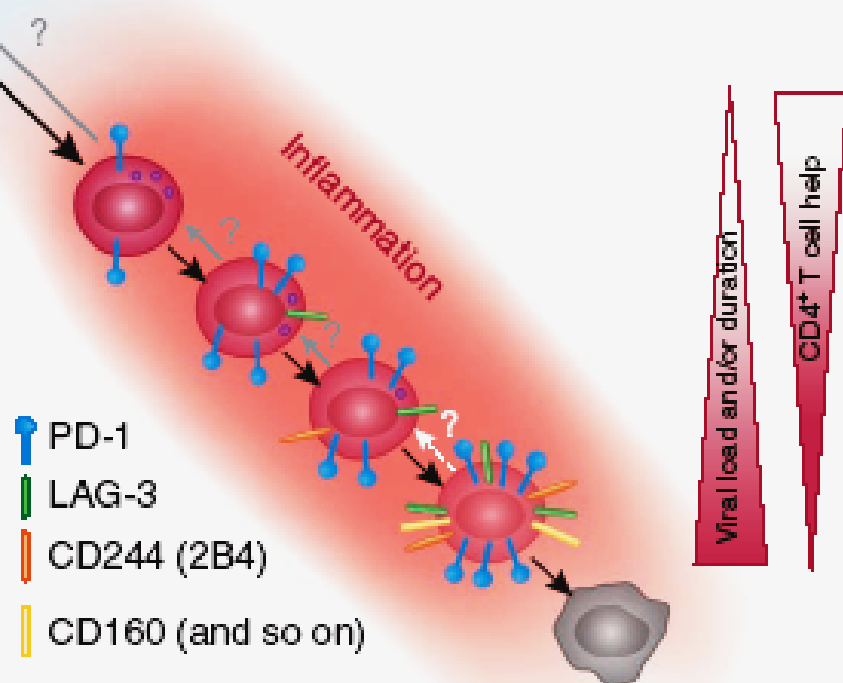
Wherry EJ

T cell exhaustion is a state of T cell dysfunction that arises during many chronic **infections** and **cancer**. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Recently, a clearer picture of the functional and phenotypic profile of exhausted T cells has emerged and T cell exhaustion has been defined in many experimental and clinical settings. Although the pathways involved remain to be fully defined, advances in the molecular delineation of T cell exhaustion are clarifying the underlying causes of this state of differentiation and also suggest promising therapeutic opportunities. Immunoregulation is centrally involved in T cell exhaustion. These negative pathways can be grouped into three main categories: cell surface inhibitory receptors (such as PD-1), soluble factors (such as IL-10), and immunoregulatory cell types (such as regulatory T cells (Treg cells) and other cells.

# T cell exhaustion during chronic activation



IFN- $\gamma$	TNF	CTL	IL-2	Proliferative potential	Apoptosis
+++	++	++/-	+/-	++	-
++	+	+	-	+	-
+/-	+/-	+/-	-	+/-	+/-
+/-	-	-	-	-	++
					+++



# Patientin WH geb. 12.6.1959; 803-4084 vom 18.7.2007

## Mamma CA, progredient

Vitamin D (25-OH)	12,6	ng/ml	30 - 80	
GSH zellulär (CD3)	290	Fimean	> 450	

### T-Zellfunktion

TCRzeta	66	% CD3	> 80	
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### ITT-Immunkontrolle

#### Basal

IL-2 Basal (TH1)	0,0	pg/ml	< 1	
IL-10 Basal (TH3)	0,6	pg/ml	< 3	
INF-gamma Basal (TH1)	0,0	pg/ml	< 1	

#### Pos. Kontrolle 1

#### PWM

IL-2 Pos. Kontrolle (TH1)	4,4	pg/ml	> 50	
IL-10 Pos. Kontrolle (TH3)	91,9	pg/ml	> 50	
INF-gamma Pos. Kontrolle (TH1)	6,5	pg/ml	> 200	

#### Antigen 1

#### Influenza

IL-2 (TH1)	0,0	pg/ml	> 15	
IL-10 (TH3)	20,8	pg/ml	< 10	
INF-gamma (TH1)	0,0	pg/ml	> 10	

**T-Zell-Exhaustion**



# Treg Dominanz

CD4 reg absolut	165	/µl	50 - 130	
CD4-Zellen regul.	16	%CD4	2 - 12	
CD4 Effektor	3	% CD4	1 - 7	
CD4 Effektor ab	30	/µl	10 - 60	
CD8-Zellen regul.	31	%CD8	60 - 80	
CD8 reg abs	126	/µl	250 - 800	

GSH in T-Zellen	-	335	←	> 450	finean
Immuntoleranz					
ITT Basal					
IL-2 Basal		0.0			pg/ml
IL-10 Basal 1		5.2			pg/ml
Interferon Bas.1		1.0			pg/ml
Antigen 1		PWM			
IL-2m Kultur 1		32.9			pg/ml
IL-10m Kultur 1		616.6	←		pg/ml
INF-γ Kultur 1		54.8			pg/ml
Antigen 2		Influenza			
IL-2m Kultur 2		5.1			pg/ml
IL-10m Kultur 2		332.2	←		pg/ml
INF-γ Kultur 2		0.0			pg/ml

# TGFbeta - IL-10

**Table 15-1 Significance and Effects of Production of IL-10 and TGF- $\beta$  by Tumor Cells**

Outcome	IL 10	TGF $\beta$
Association of systemic levels and poorer prognosis	+	+
Inhibition of growth of T cells	-	+
Inhibition of T cell differentiation	+	+
Inhibition of production of cytokines	+	+
Promotion of anergy of T cells	-	+
Skewing toward Th2 responses	+	+
Disruption of antigen presentation	+	+
Downregulated expression of co-stimulatory molecules	+	+
Increased resistance of tumor cells to lysis	+	-
Dysregulation of cytolytic effector cells	+	+

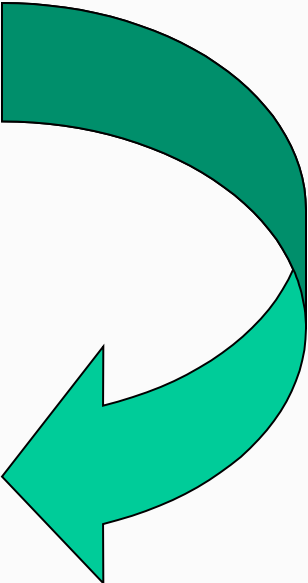
From: Mechanisms of Immunosuppression

# *TH1-relevante Mikronährstoffe*

- **Vitamin D**
- **Glutathion**
- **Selen**
- **Zink**
- **B-Vitamine**
- **Cimetidin**
- **Immunpilze**
- **Biobran/MGN3**
- **Mistel**
- **DCA**
- **Delimmun**
- **Arginin**

# T-Select: IFNg-Induktion

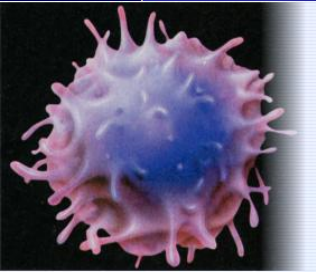
Immunologie		
Immuntoleranz		
ITT Basal		
IL-2 Basal	0.0	pg/ml
IL-10 Basal 1	1.7	pg/ml
Interferon Bas.1	0.0	pg/ml
Antigen 1	PWM	
IL-2m Kultur 1	639.6	pg/ml
IL-10m Kultur 1	228.9	pg/ml
INF-g Kultur 1	>1000.0	pg/ml
Antigen 2	Zink	
IL-2m Kultur 2	1.0	pg/ml
IL-10m Kultur 2	1.7	pg/ml
INF-g Kultur 2	2.0	pg/ml
ITT: Antigen 3	Vitamin C	
IL-2m Kultur 3	7.3	pg/ml
IL-10m Kultur 3	7.2	pg/ml
INF-g Kultur 3	108.3	pg/ml
Antigen 4	Thymus	
IL-2m Kultur 4	16.4	pg/ml
IL-10m Kultur 4	12.8	pg/ml
INF-g Kultur 4	153.2	pg/ml
Antigen 5	Glutathion	
IL-2m Kultur 5	0.0	pg/ml
IL-10m Kultur 5	1.6	pg/ml
INF-g Kultur 5	6.3	pg/ml
Antigen 6	Mistellektin	
IL-2 Kultur 6	0	pg/ml
IL-10 Kultur 6	2	pg/ml
INFg Kultur 6	3	pg/ml



# T-Select: IL10 Reduktion durch Vitamin C

T-Zellfunktion	
T-Select	
<b>Basalkultur</b>	
Basal + IL2	0
Basal + IL10	556
Basal + IFN	5
<b>Kontrolle</b>	<b>PWM</b>
Kontrolle IL-2	253
Kontrolle IL-10	666
Kontrolle IFN	1.435
<b>Modulator 2</b>	<b>Thymus</b>
Modulator 2 + IL-2	2
Modulator 2 + IL-10	502
Modulator 2 + IFN	12
<b>Modulator 3</b>	<b>Mistel</b>
Modulator 3 + IL-2	0
Modulator 3 + IL-10	304
Modulator 3 + IFN	3
<b>Modulator 4</b>	<b>Glutathion</b>
Modulator 4 + IL-2	0
Modulator 4 + IL-10	316
Modulator 4 + IFN	2
<b>Modulator 5</b>	<b>Vitamin C</b>
Modulator 5 + IL-2	0
Modulator 5 + IL-10	4
Modulator 5 + IFN	0
<b>Modulator 6</b>	<b>H15 Gufic</b>
Modulator 6 + IL-2	0
Modulator 6 + IL-10	269
Modulator 6 + IFN	35

# Funktionstests



## T-Zellen

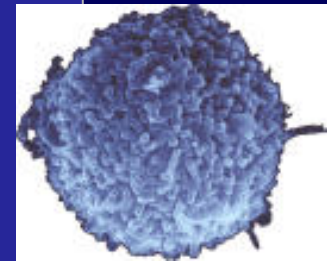
ITT Zytokin-LTT

T-Select

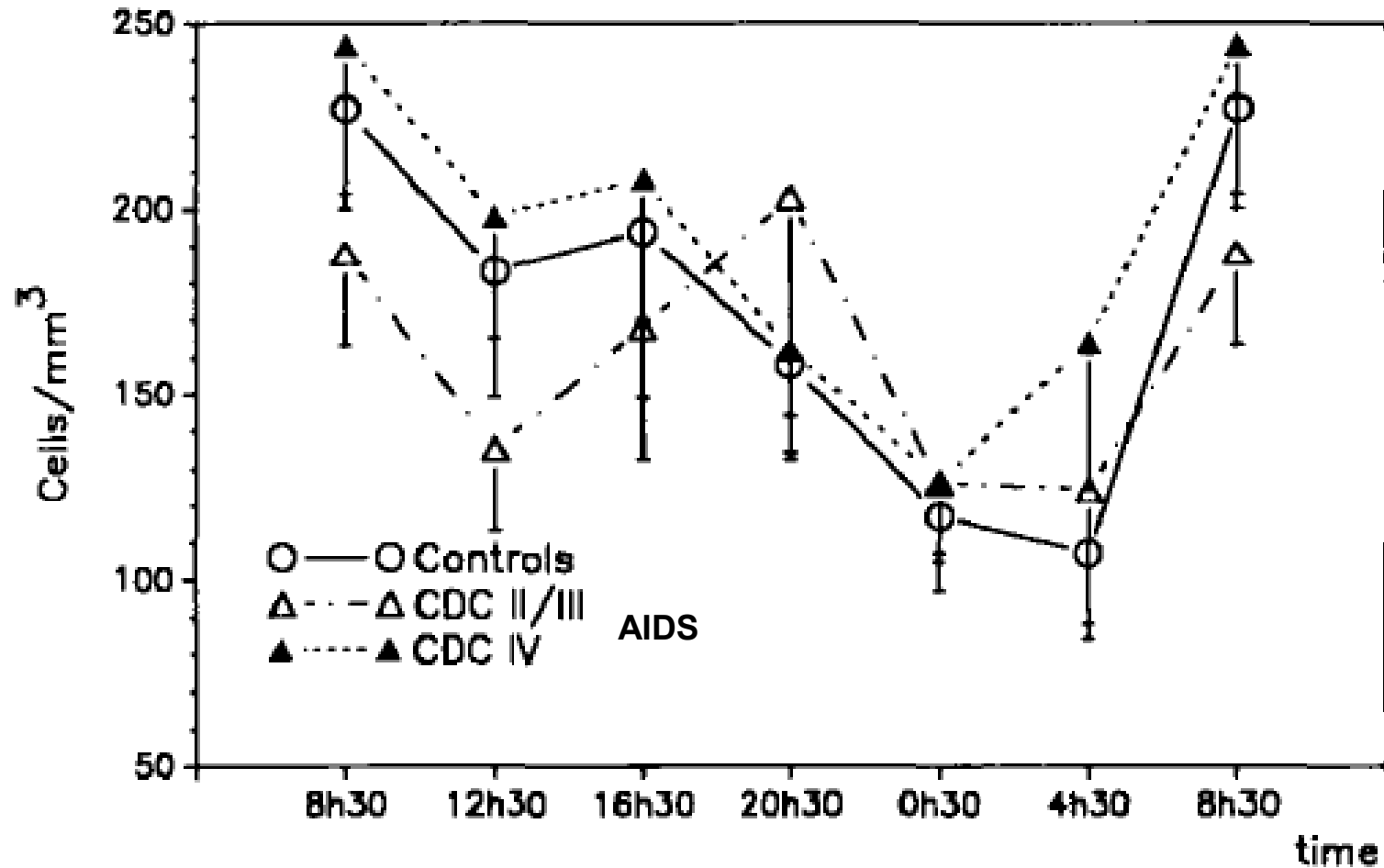
## NK-Zellen

- NK-Check

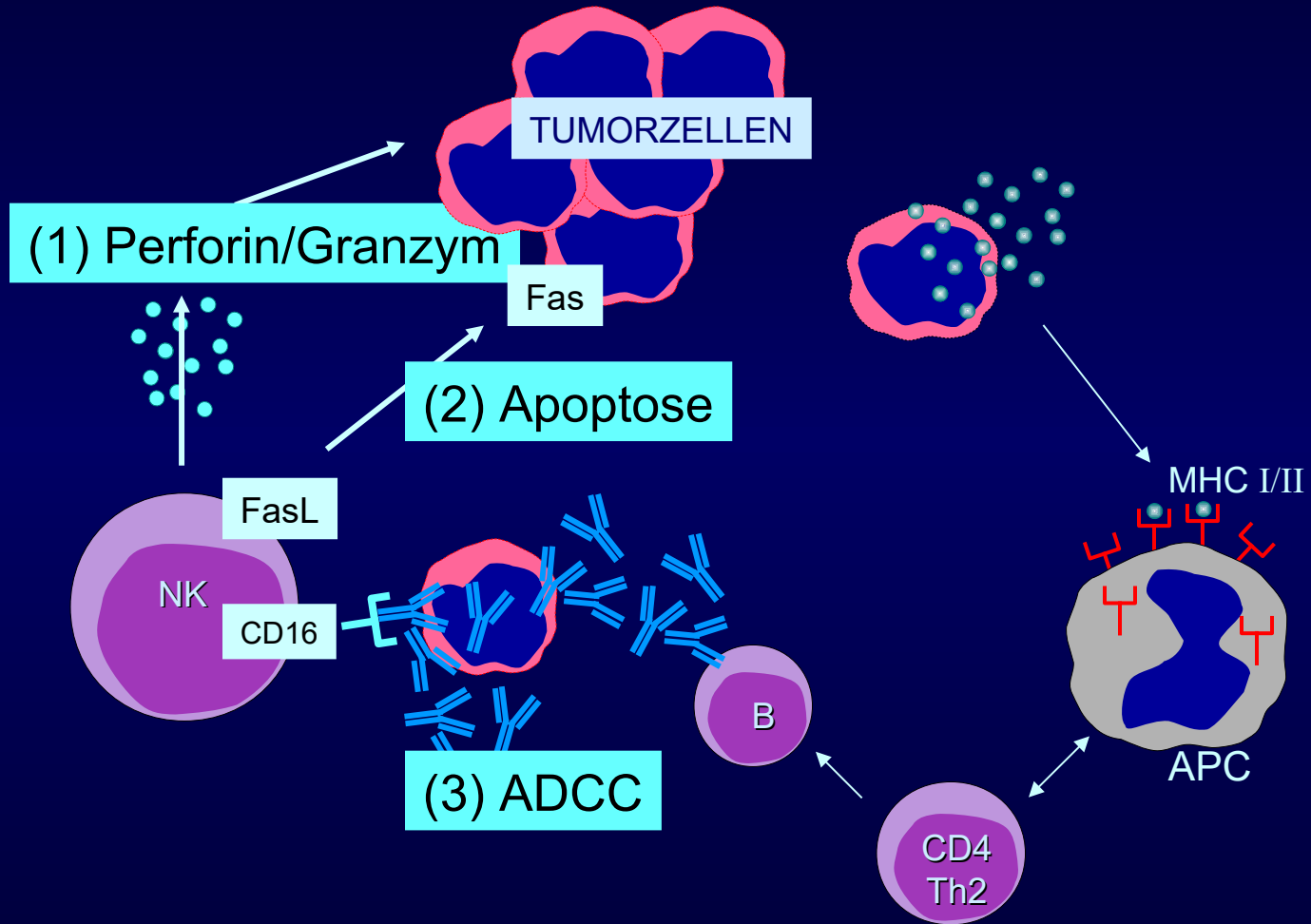
- NK-Select



# Tagesrhythmik der NK-Zellen im peripheren Blut



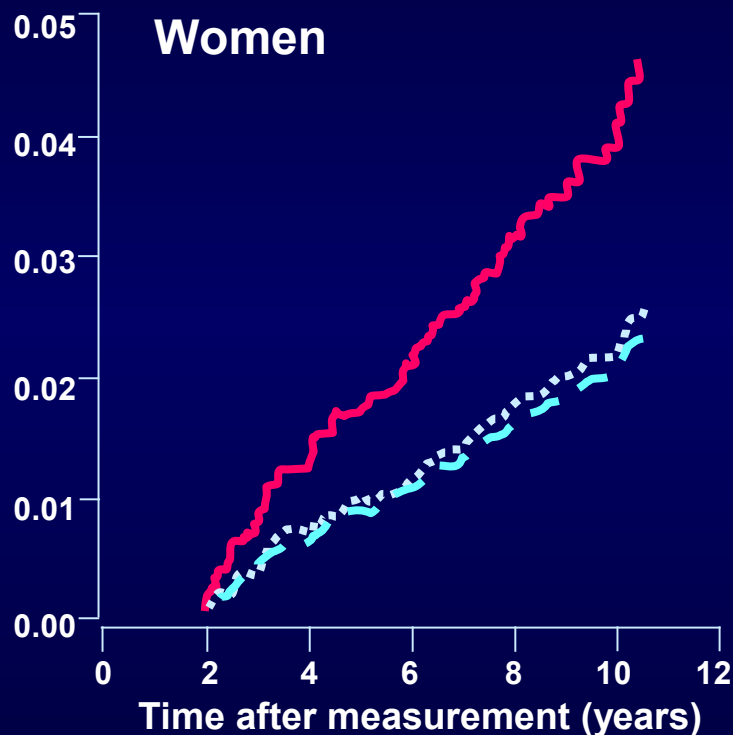
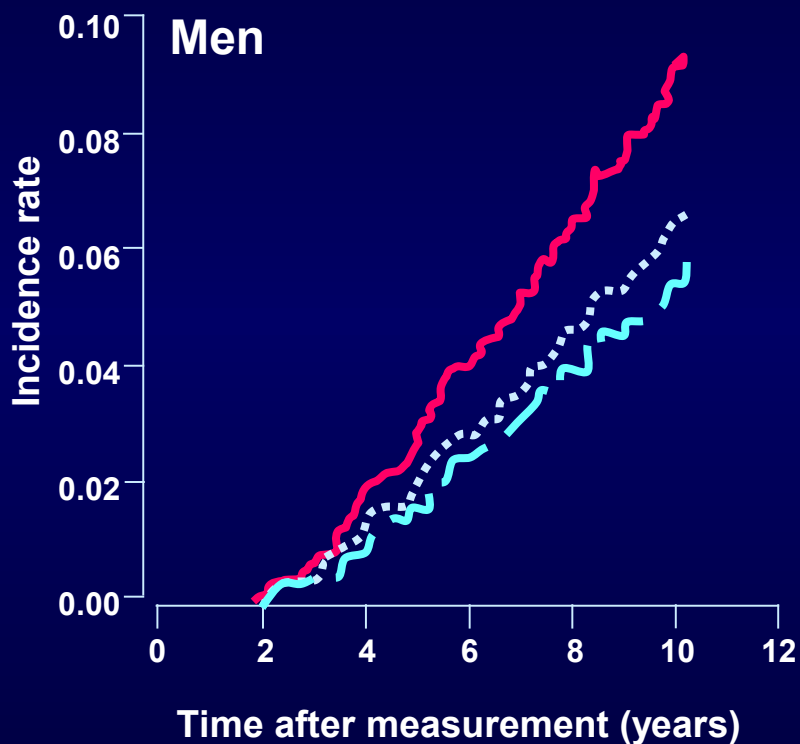
# Die drei Killing-Mechanismen der NK-Zellen





# NK Activity of Lymphocytes and Cancer Incidence: An 11 year follow up.

8552 individuals older than 40 years



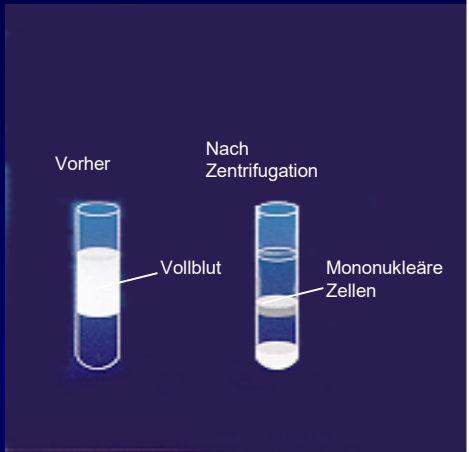
— High NK Activity  
- - - Medium  
— Low

Categorised by tertiles. Men – low: ≤42%; medium: 43 - 58%; high > 58%  
Women – low: 34%; medium: 35 - 51%; high: 51%

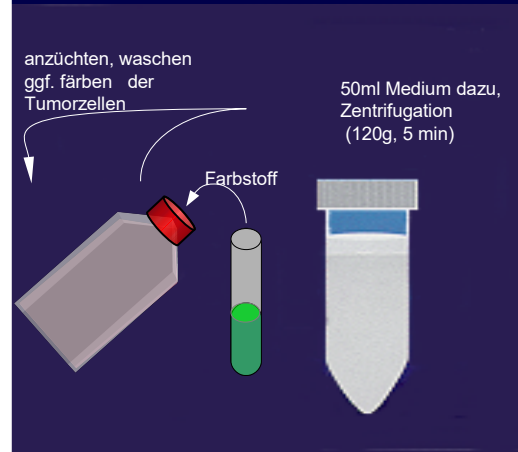
# NK-Check®: Abtötung von Tumorzellen

## 1. Probenvorbereitung

Dichtezentrifugation mittels Ficoll

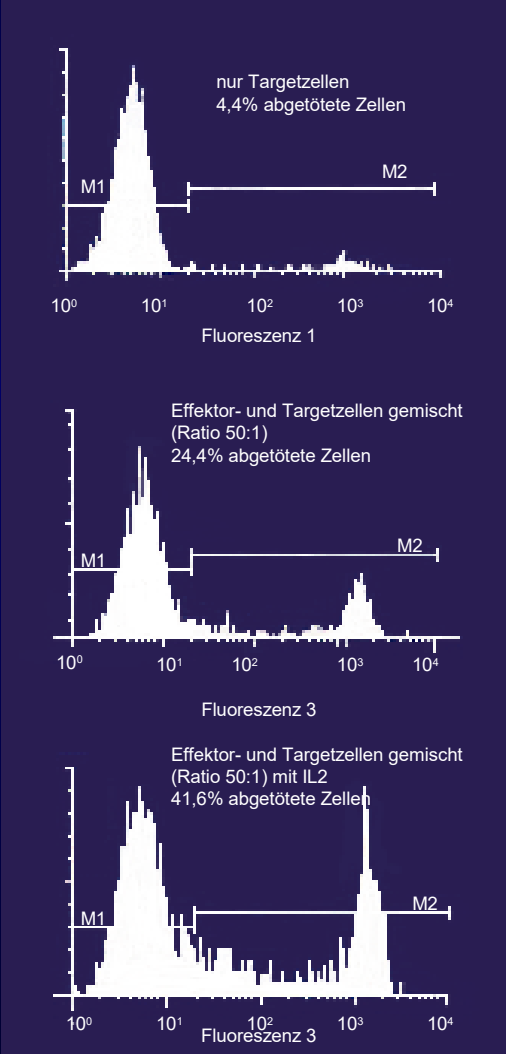


## 2. Präparation der Tumorzell-Line

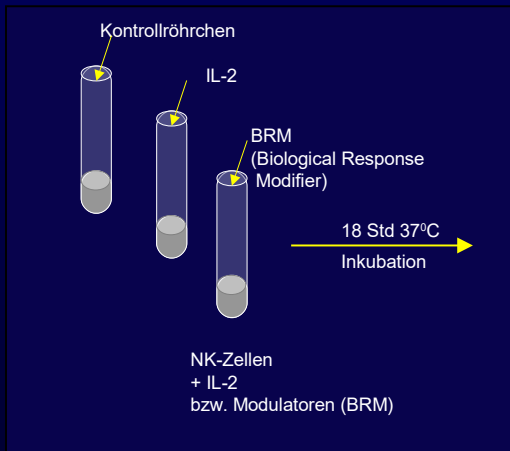


## 5. Auswertung

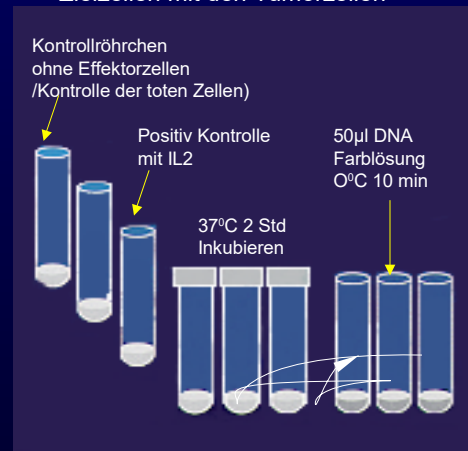
Bestimmung der abgetötete Tumorzellen



## 3. NK-Assay / Immunmodulatoren



## 4. Funktionstest gemeinsame Inkubation der Zielzellen mit den Tumorzellen



## Functional significance of the activation-associated receptors CD25 and CD69 on human NK-cells and NK-like T-cells

Johannes Clausen, Birgit Vergeiner, Martina Enk, Andreas L. Petzer, Günther Gastl, Eberhard Gunsilius

Tumor Biology & Angiogenesis Laboratory, Division of Hematology & Oncology, University Hospital, Innsbruck, Austria

Received: November 14, 2001 · Accepted: July 29, 2002

### Abstract

The application of autologous ex-vivo expanded cytotoxic lymphocytes to cancer patients may help to control minimal residual disease. However, the number of effector cells and the resulting antitumoral activity that can be generated *in vitro* are remarkably variable. Thus, we separately assessed the proliferative and cytotoxic potential of CD56<sup>+</sup>CD3<sup>-</sup> natural killer (NK) and CD56<sup>-</sup>CD3<sup>+</sup> T-cells in relation to their expression of CD25, CD69, and CD16 *in vitro*. Two-week lymphocyte cultures from peripheral blood (n = 51) and from G-CSF-mobilized progenitor cell harvests (n = 11) were performed repeatedly from 14 women with breast cancer throughout conventional- and high-dose chemotherapy. A large proportion of CD25<sup>+</sup> cells on day 7 of the culture predicted high expandability (r = 0.69, p < 0.00001), while elevated expression of CD69 predicted augmented cytotoxicity (r = 0.72; p = 0.00001) and low expandability (r = -0.69, p < 0.00001). CD25 and CD69 expression were inversely correlated (r = -0.8, p < 0.0001). CD16 expression was not suited to predict functional properties. Additionally, NK-cells were sorted by FACS according to CD25 versus CD69 expression. In a [<sup>3</sup>H]thymidine incorporation assay the CD25<sup>-</sup> NK-cell fraction exhibited a higher proliferation rate than did the CD69<sup>+</sup> fraction in all of three experiments. Together, our data suggest that CD69 is a useful marker for cytotoxic activity of NK cells, whereas proliferative potential is indicated by CD25 expression. These findings should help optimizing the ex-vivo generation of large numbers of cytotoxic effector cells for immunotherapy.

# Patientin 68J

01073723, 21.09.2004

**Diagnose:  
Mamma-Ca**

## IMMUNOLOGIE

CD80 auf AP C relativ	9	% AP C	2 - 5	
CD80 auf AP C	146	/µl	30 - 75	
CD86 auf AP C relativ	14	% AP C	15 - 25	
CD86 auf AP C	220	/µl	250 - 500	
T4 Zellen Costimulation CD28 relativ	66	% CD4	98 - 100	
T8 Zellen Costimulation CD28 relativ	13	% CD8	> 50	
Ratio Suppressor/Zytotoxische	4			
Leukozyten	5,8	/nl	4.0 - 10.0	
Granulozyten	1900	/µl	2400 - 7400	
Granulo %	34	%	42 - 75	
Monozyten	2300	/µl	140 - 800	
Mono %	39	%	2 - 14	
Lymphozyten	1600	/µl	1100 - 4000	
Lympho %	27	%	20 - 40	
B-ZELLEN (CD19)	77	/µl	120 - 630	
B(CD19) %	5	% Lympho	7 - 21	
T-ZELLEN (CD3)	1081	/µl	920 - 2580	
CD3 %	68	% CD3	60 - 84	
CD4-Helferzellen	347	/µl	550 - 1660	
CD4 %	22	% CD3	32 - 60	
CD4-Zellen (CD28+)	216	/µl	> 550	
CD8-Zellen	711	/µl	380 - 930	
CD8 %	44	% CD3		
CD8-Zellen (CD28+)	97	/µl		
CD4/CD8 RATIO	0,49			
CTL (cytotoxische T-Zellen)	171	/µl		
CTL %	16	% CD3		
akt.T-ZELLEN (CD25/IL-2)	98	/µl		
akt.T(CD25) %	9	% CD3		
NK-ZELLEN (CD16/CD56)	223	/µl	210 - 740	
akt.NK-ZELLEN (CD25)	29	/µl	0 - 10	
akt.NK(CD25) %	6	% NK	0 - 4	
NK-Zellen %	14	% Lympho	6 - 29	
NK-Zellen regulatorisch	10	% NK	5 - 15	
NK-Zellen zytotoxisch	90	% NK	85 - 95	
NKzyt:NKreg Ratio	9	Ratio	< 13	

**NK-Zellen  
-aktiviert: CD25  
-zytotoxisch: CD16**



# Patientin TI

Geb.27.06.1965

740-0541 vom 22.6.2011

## Diagnosen:

Mamma-CA, metastasierend

### T-Zellfunktion

#### ITT-Immunkfunktion

##### Basal

IL-2 Basal (TH1)	0,0	pg/ml	< 1
IL-10 Basal (TH3)	1,4	pg/ml	< 3
INF-gamma Basal (TH1)	0,0	pg/ml	< 1

##### Pos. Kontrolle 1

##### PWM

IL-2 Pos. Kontrolle (TH1)	940,1	pg/ml	> 100
IL-10 Pos. Kontrolle (TH3)	2486,9	pg/ml	> 50
INF-gamma Pos. Kontrolle (TH1)	1924,0	pg/ml	> 200

##### Antigen 1

##### Influenza

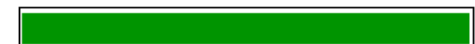
IL-2 (TH1)	40,0	pg/ml	> 15
IL-10 (TH3)	300,5	pg/ml	< 10
INF-gamma (TH1)	28,2	pg/ml	> 10

### NK-Zellfunktion

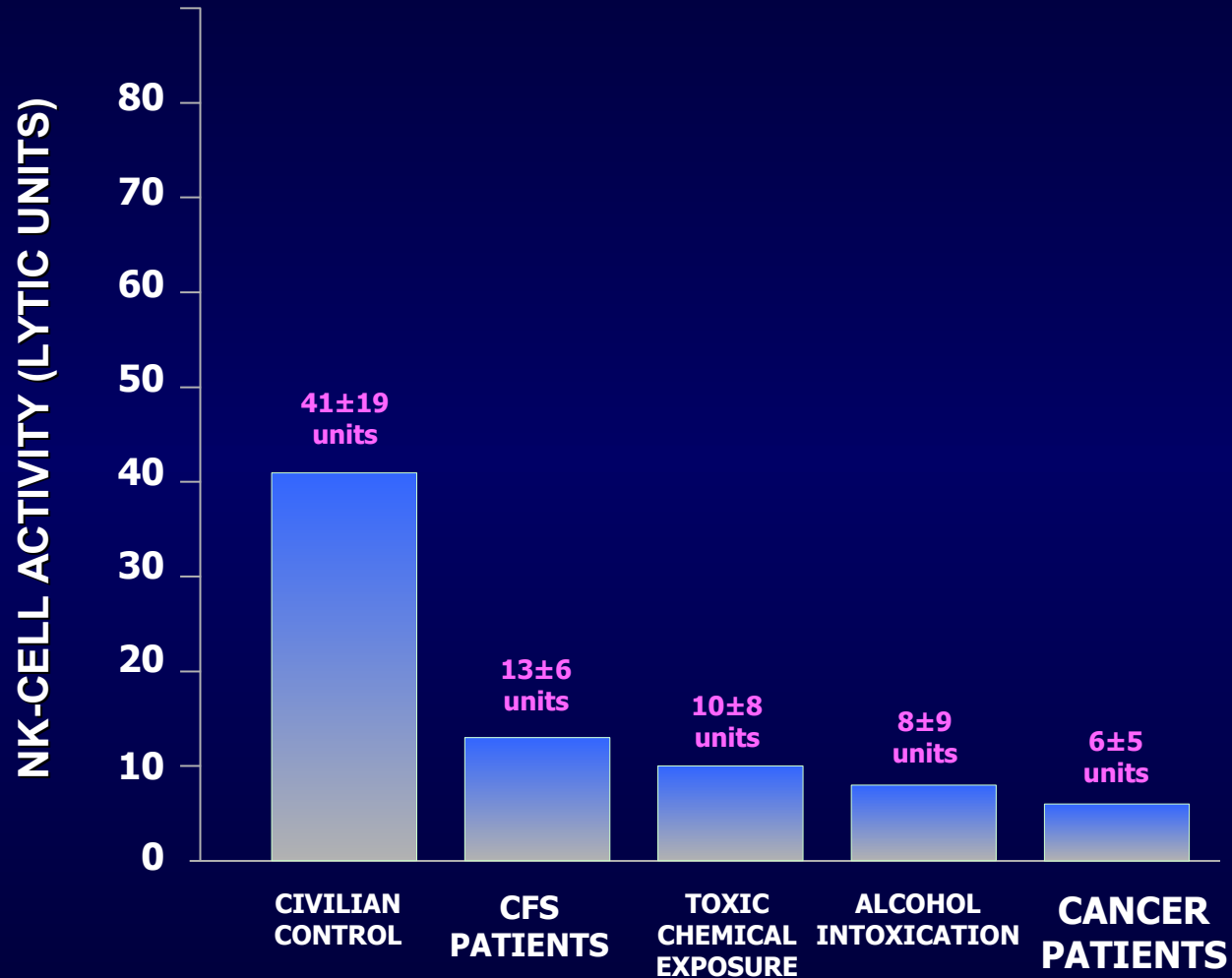
#### NK-Check®

NK/Ctx: basal	23	% K562	> 20
NK/Ctx: IL-2 stim	23	% K562	> 35
NK-Zellen CD69 basal	6	% NK	
NK-Zellen CD 69 IL2	22	% NK	
NK-Zellen (relativ)	15	% Lympho	6 - 29

**NK-Aktivität reduziert**



# NK-Cell Activity in different Patient Groups



**Patient MH**

Geb. 19.09.1942

830-2549 vom 29.8.2012

**Diagnosen:**

Prostata CA


**6-8**

## NK-Zellfunktion

NK-Zellen (absolut)	186	/ $\mu$ l	100 - 600
NK-Zellen (relativ)	18	% Lympho	6 - 29

### NK-Zellfunktion

#### NK-Check®

NK/Ctx: basal	8		% K562	> 20
NK/Ctx: IL-2 stim	21		% K562	> 35
NK-Zellen CD69 basal	2		% NK	
NK-Zellen CD 69 IL2	47		% NK	

# Patientin WH geb. 125.6.1959; 803-4084 vom 18.7.2007

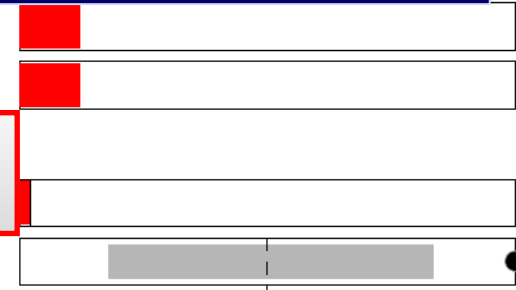
## Mamma CA, progredient

### NK-Zellfunktion

#### NK-Check®

NK/Ctx: basal	18	% K562	> 20
NK/Ctx: IL-2 stim	18	% K562	> 35
NK-Zellen CD69 basal	2	% NK	
NK-Zellen CD 69 IL2	2	% NK	
NK-Zellen (relativ)	35	% Lympho	6 - 29

### NK-Zell-Anergie





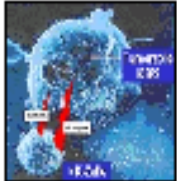
# ***Negative Einflüsse auf die NK-Aktivität***

- **physischer und psychischer Stress**  
**(Cortisol, Adrenalin, Noradrenalin)**
- **Depressionen**
- **ROS, NOS (Peroxynitrit; Glutathion)**
- **körperliche Inaktivität**
- **Körperliche Überlastung**
- **Vitamin-/Vitalstoffmangel**
- **Hypothyreose**
- **Treg: TGF $\beta$ , IL-10**

# *Bewährte Immunmodulatoren*

- **Biobran (MGN-3)**
- **Immunpilze/AHCC**
- **Thymus**
- **Mistel**
- **Vitamin C** hochdosiert
- **Cimetidin**
- **Vitamin D**
- **Levamisol**
- **Arginin, Lysin**
- **Zink, Selen**
- **Echinacea**
- **Glutathion/NAC**
- **Glutamin**
- **Curcumin, EGCG**
- **Resveratrol**
- **Probiotika**
- **B-Vitamine**
- **β-Blocker**

# NK-Select



## NK-Zellfunktion

**NK-SELECT®**

**NK-Check®**

NK/Ctx: basal	16	% K562	> 20
NK/Ctx: IL-2 stim	23	% K562	> 35
NK-Zellen CD69 basal	3	% NK	
NK-Zellen CD 69 IL2	41	% NK	

### MODULATOR 1

NK-Zellen CD 69 Mod 1 19 ← % NK

### MODULATOR 2

NK-Zellen CD 69 Mod 2 32 ← % NK

### MODULATOR 3

NK-Zellen CD 69 Mod 3 6 % NK

### MODULATOR 4

NK-Zellen CD 69 Mod 4 10 % NK

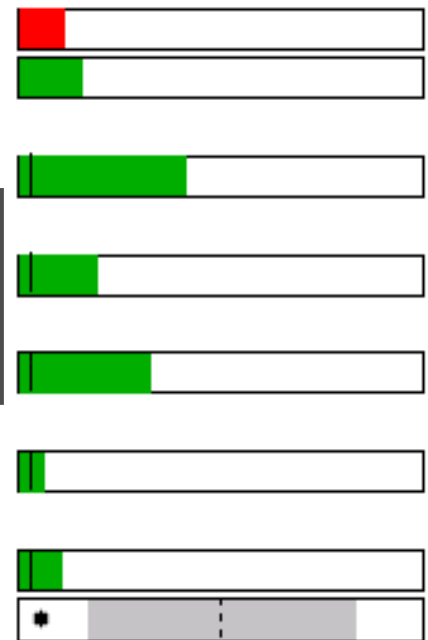
NK-Zellen (relativ) 2 % Lympho 6 - 29

**MGN3**

**Immunpilze**

**Vitamin C**

**Mistel**



# NK-Select

## Patientin IB

Geb.26.09.1939

830-1972 vom 28.7.2010

### Diagnosen:

2010 Colon-CA (OP) + Lebermetastase (Hyperthermie)

- NK-Funktion – MGN3

- Behandlungsindikation

### NK-SELECT®

NK Zellen cytotoxisch 89 % NK 85 - 95

NK Zellen regul. 11 % NK 5 - 15

### NK-Check®

NK/Ctx: basal 29 % K562 > 20

NK/Ctx: IL-2 stim 29 % K562 > 35

NK-Zellen CD69 basal 8 % NK

NK-Zellen CD 69 IL2 58 % NK

### MODULATOR 1

### MGN3

NK-Zellen CD 69 Mod 1 59 % NK ←

### MODULATOR 2

### Immunpilze

NK-Zellen CD 69 Mod 2 47 % NK ←

### MODULATOR 3

### Vitamin C

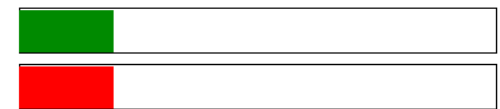
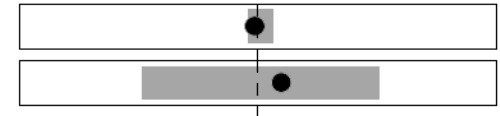
NK-Zellen CD 69 Mod 3 21 % NK

### MODULATOR 4

### Mistel

NK-Zellen CD 69 Mod 4 28 % NK

NK-Zellen (relativ) 9 % Lympho 6 - 29



**Therapie > Immunrestitution  
T – und NK-Zellfunktion**

**ITT-Immunkfunktion**

Basal	basal	basal	
IL-2 Basal (TH1)	0,0	0,0	pg/ml
IL-10 Basal (TH3)	0,0	0,7	pg/ml
INF-gamma Basal (TH1)	0,0	0,2	pg/ml
<b>Pos. Kontrolle 1</b>	<b>PWM</b>	<b>PWM</b>	
IL-2 Pos. Kontrolle (TH1)	38,6	33,5	pg/ml
IL-10 Pos. Kontrolle (TH3)	49,6	192,1	pg/ml
INF-gamma Pos. Kontrolle (TH1)	232,6	261,0	pg/ml

Antigen 1	Influenza	Influenza	
IL-2 (TH1)	33,0	7,9	pg/ml
IL-10 (TH3)	22,3	4,7	pg/ml
INF-gamma (TH1)	28,0	6,1	pg/ml

**NK-Zellfunktion**

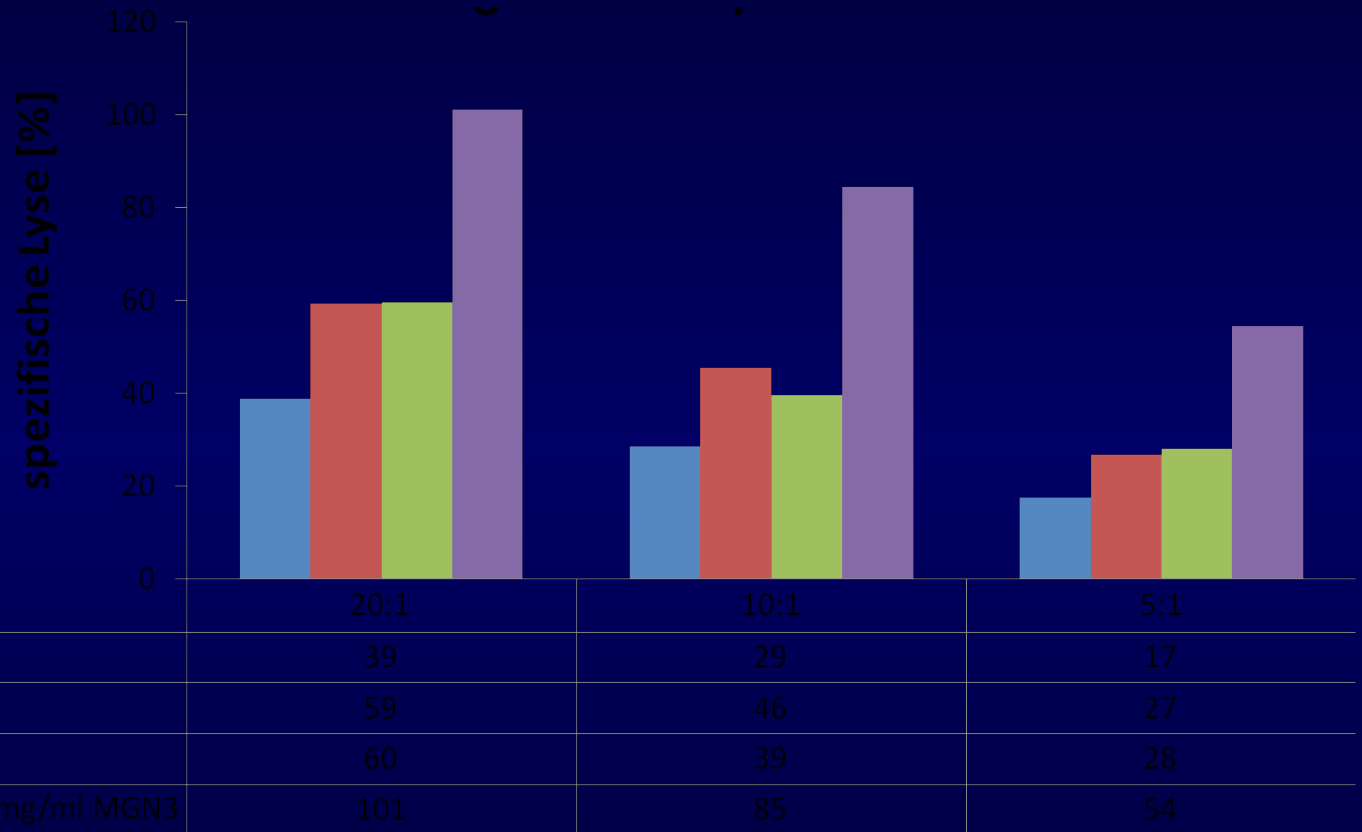
**NK-Check®**

NK/Ctx: basal	53	18	% K562
NK/Ctx: IL-2 stim	53	18	% K562
NK-Zellen CD69 basal	1	7	% NK
NK-Zellen CD 69 IL2	47	24	% NK
NK-Zellen (relativ)	21	13	% Lympho



# NK Aktivität gegenüber K 562 Zellen II

## Biobran = MGN3



# „Rationelles“ Erstprogramm

Blutbild

(TU-Marker), uPAR

Vitamin D

Glutathion<sub>zell</sub>

CoQ10

Homocystein

ATP, Nitrotyrosin

Ferritin

Ω3-Fettsäuren

Selen

TNF $\alpha$ , IL-8, IFN $\gamma$

IL12, IL-17

IL-10, TGF $\beta$

sIL2r

TU-Immunprofil

T-Zellaktivität

T-Exhaustion (PD-1)

Treg-Zellen

Zytokin-LTT

NK-Zytotoxizität

Cortisol/DHEA

Serotonin (5HIES)

# „Rationelles“ Verlaufsprogramm

**Blutbild**

**TU-Marker, uPAR**

**Vitamin D**

**ATP, Glutathion**

**TNF $\alpha$ , IL-8,**

**IL-10, TGF $\beta$ ,**

**IL12, IFN $\gamma$**

**sIL2r**

**VEGF, MIF**

**Treg-Zellen**

**T-Exhaustion (PD-1)**

**Zytokin-LTT**

**T-Modulatoren**

**NK-Zytotoxizität**

**NK-Modulatoren**

**DTC**



Time to say goodbye

