Identification of CD4+ T cell epitopes specific for the breast cancer associated antigen NY-BR-1

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dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

50 Years – Research for A Life Without Cancer

Introduction

- **Aim: Licensing patient's immune system to defend cancer**

- **Include different immune cells (CD8, CD4 T cells), inhibitory and stimulatory molecules**

   T-helper-1-cell cytokines drive cancer into senescence
   Braumüller et al., Nature 2013

   CD4+ T cells predict breast cancer survival
   Gu-Trantien et al., J Clin. Invest. 2013

- **Antigen-specific CD4^{+} T cells in breast cancer**

- **Target antigen: NY-BR-1**

NY-BR-1 is expressed in breast cancer cells

1. NY-BR-1 mRNA expression

breast tissue, prostate, testis

overexpressed in **80% of all tested breast cancers**
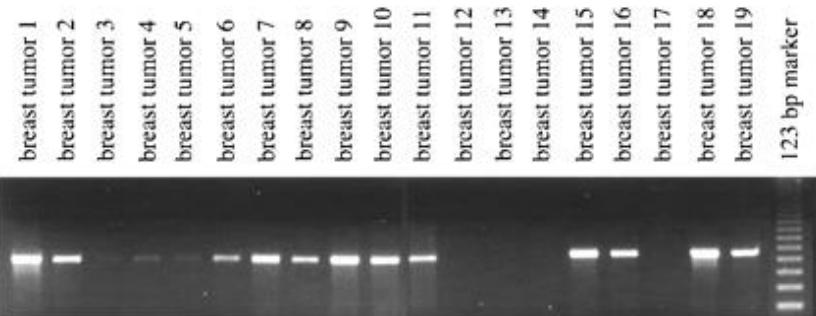
Jäger, Cancer Res. 2001 Mar 1;61(5):2055-61

2. NY-BR-1 protein expression

solely in epithelial cells of the breast

60% of invasive ductal mammary carcinomas
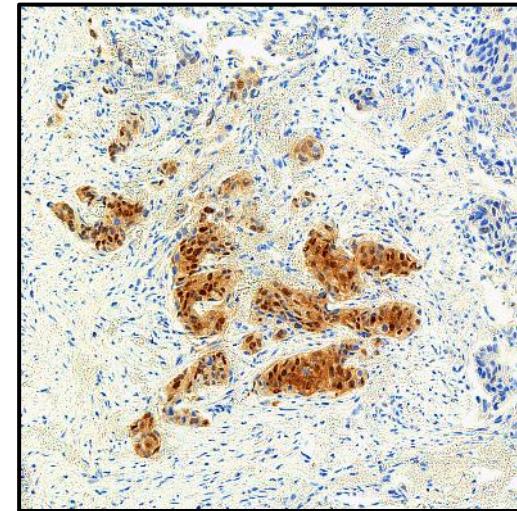
NY-BR-1

T cell epitope restriction

| MHC-I	MHC-II
CD8$^+$ T cells: =>Direct targeting of MHC-I$^+$ tumor cells	CD4$^+$ T cells: => targeting of tumor cells (1) Licensing of DCs to effectively prime CTLs (2) Direct stimulation of CTLs (3) Tumor stroma targeting (TAMs)

NY-BR-1 class I epitopes:
Jäger et al., 2005, Wang et al., 2006

NY-BR-1 class II epitopes:
Gardyan et al., 2015

# Screening of a NY-BR-1-specific library leads to the identification of NY-BR-1 candidate epitopes

library peptide (20mer)	predicted epitope (15mer)	prediction score[*] (HLA-DRB1*0301)
#12	**BR1-88**	34
#156	**BR1-1245**	13
#169	**BR1-1347**	22

| library peptide (20mer)	predicted epitope (15mer)	prediction score[*] (HLA-DRB1*0401)
#68	**BR1-537**	26
#97/82	**BR1-656/-775**	26
#156	**BR1-1242**	20

*SYFPEITHI database

Detection of epitope specific T cell-response in HLA-tg mice vaccinated with a NY-BR-1 encoding expression vector


A: DR3tg mice
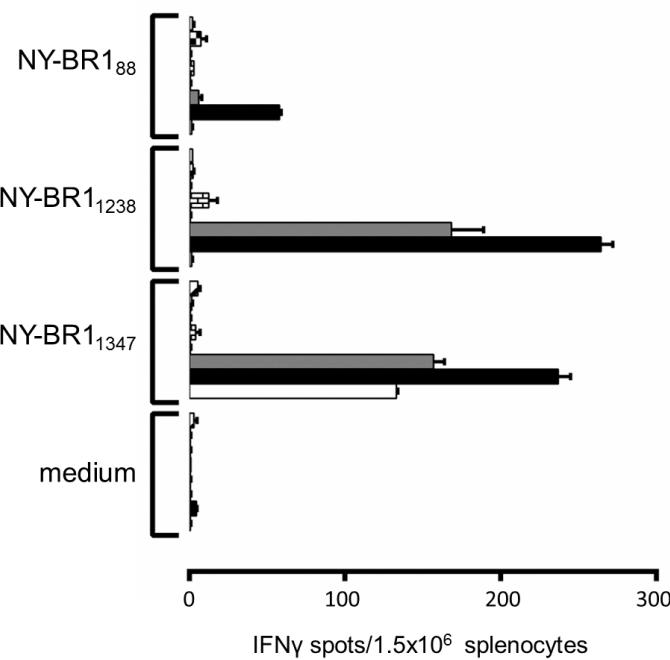
B: DR4tg mice
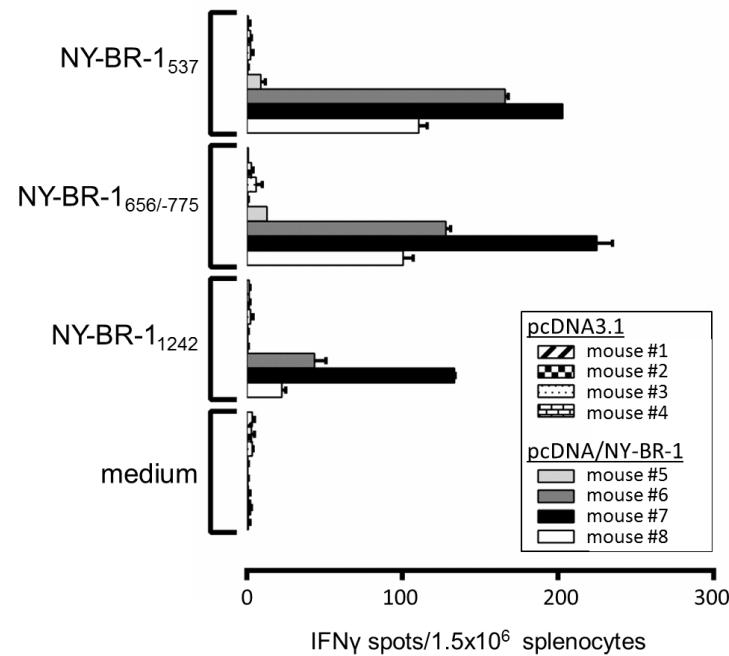
immunization: DNA
read-out: 15 mer peptides (putative epitopes)

detection of epitope ✔

Confirmation of HLA-DR-restriction of murine NY-BR-1-specific CD4$^+$ T cell lines

A: T cell lines form DR3tg mice
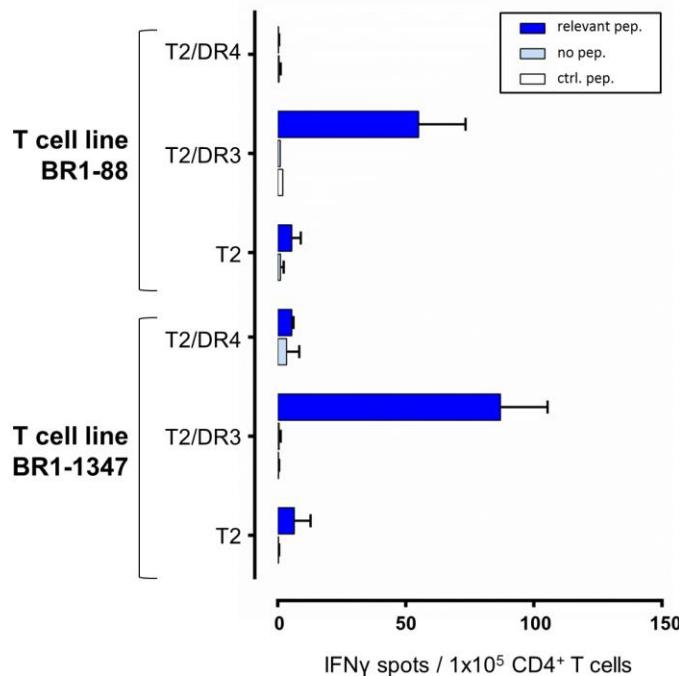
B: T cell lines from DR4tg mice
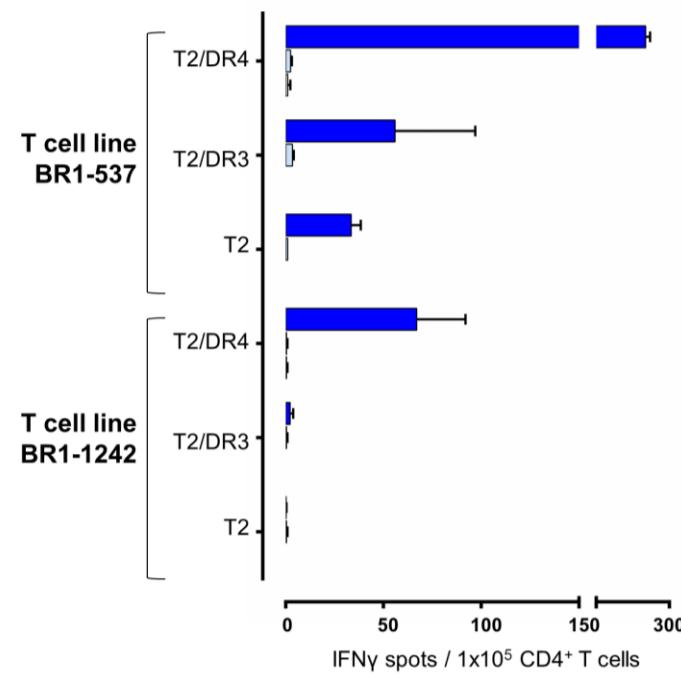
T2: human, class II negative tumor cell line (TxB) transfectants express DR4 or DR3

HLA restriction ✓

NY-BR-1-specific CD4+ T cell epitopes are processed by human cells

A: DR3tg

- T cell line BR1-88
- T cell line BR1-1347

B: DR4tg

- T cell line BR1-537
- T cell line BR1-1242

IFNγ spots / 1x10⁵ CD4+ T cells
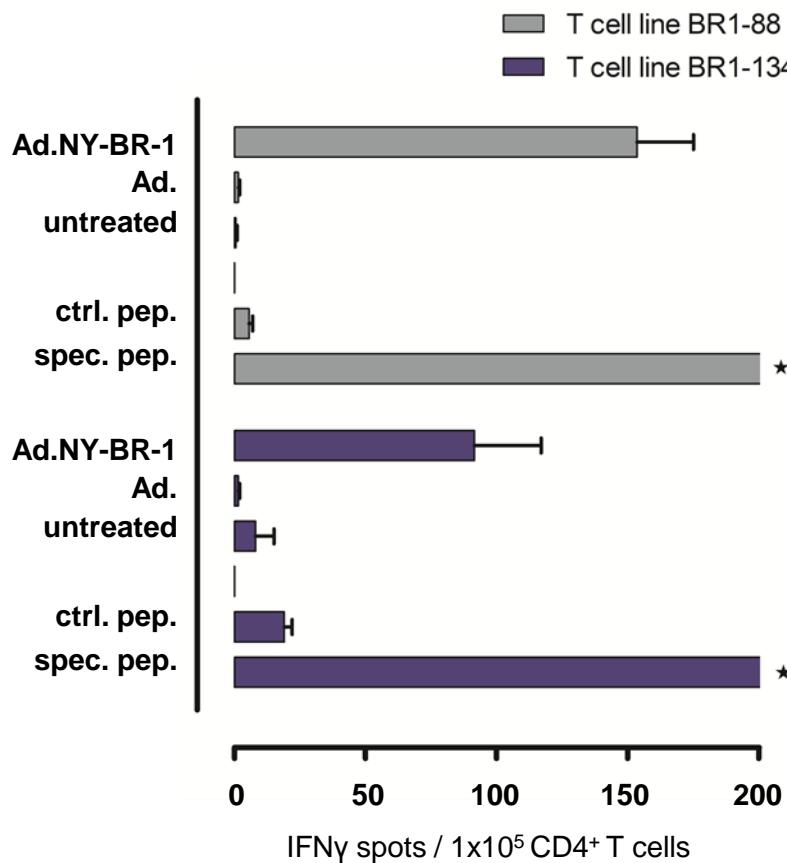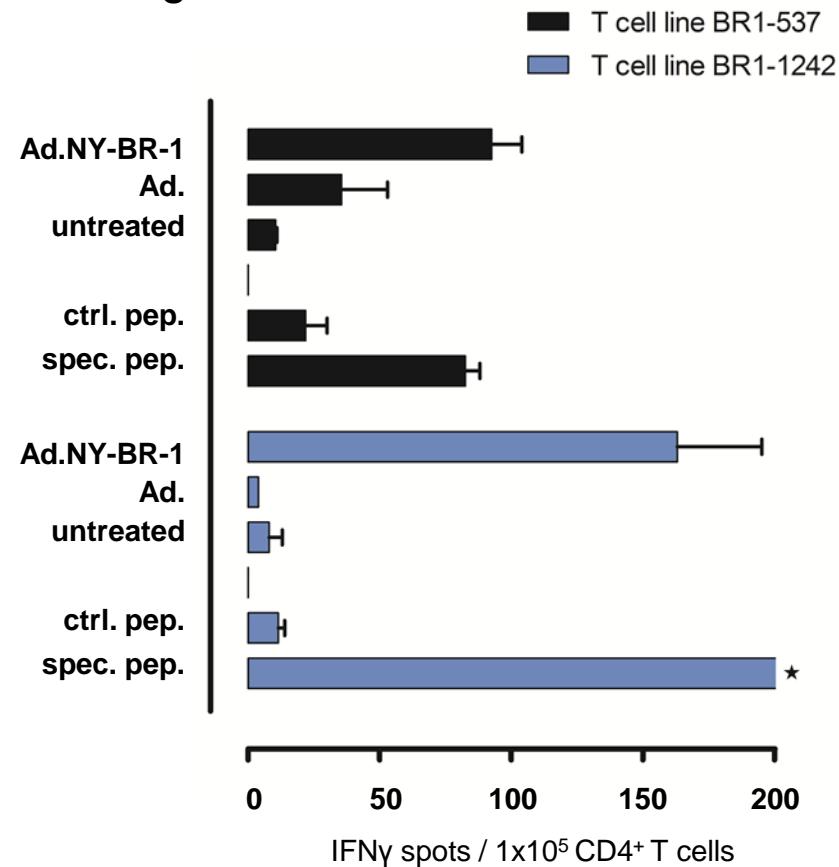
* over-saturated wells

natural processing in human cells ✔

Patients' characteristics
(selected for NY-BR-1 expression and HLA type)

| Patient code	BC#1	BC#2	BC#3	BC#4	BC#5	BC#6	BC#7
Age at first diagnosis	67	68	71	88	84	48	52
Primary tumor	no	yes	yes	yes	yes	yes	yes
Metastasis	Lung, liver	no	no	no	no	no	no
TNM	T2 N0 M1	T2 N3 M0	T1 N1 M0	T2 N2 M0	T2 N1 M0	T4 N0 M0	T2 N1 M0
ER [2]	4/12	8/8	8/8	8/8	8/8	8/8	8/8
PgR [2]	12/12	7/8	8/8	8/8	8/8	8/8	8/8
Her2/neu	0	0	1	1	1	0	1
Phenotype	ILC	Luminal-B	Luminal-A	Luminal-B	Luminal-A	Luminal-B	Luminal-A
HLA-DRB1*	0301	0301	0301	0404	0402	0401	0405
Chemotherapy before blood draw	Vinorelbin	no	n.a.	no	no	no	no
Irradiation before blood draw	11 years	no	no	no	no	no	no
Biopsy before blood draw							
NY-BR-1 intensity [3]	++	++	++	++	++	+	++
NY-BR-1$^+$ cells (frequency)	80%	50%	90%	70%	90%	40%	90%

NY-BR-1 expression in tumors from breast cancer patients (BC) selected for HLA-type
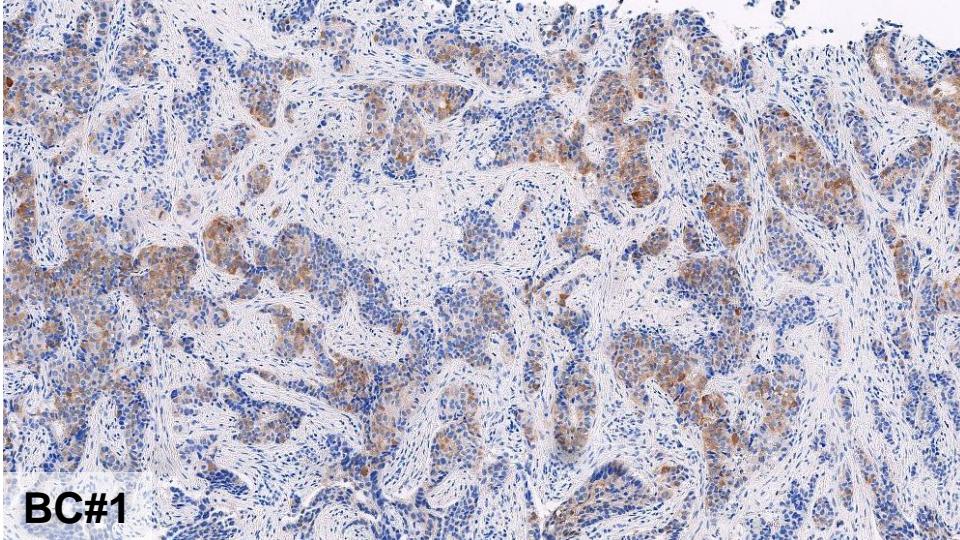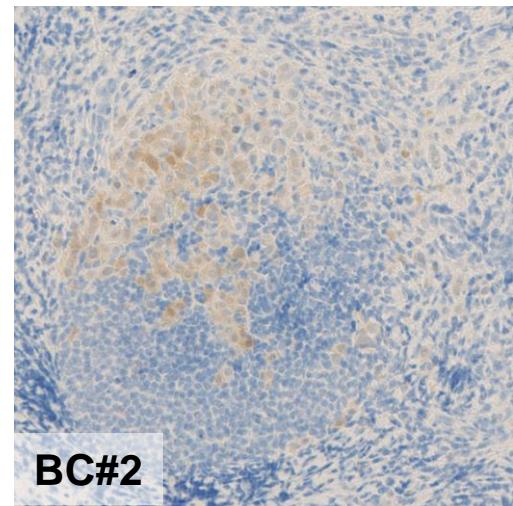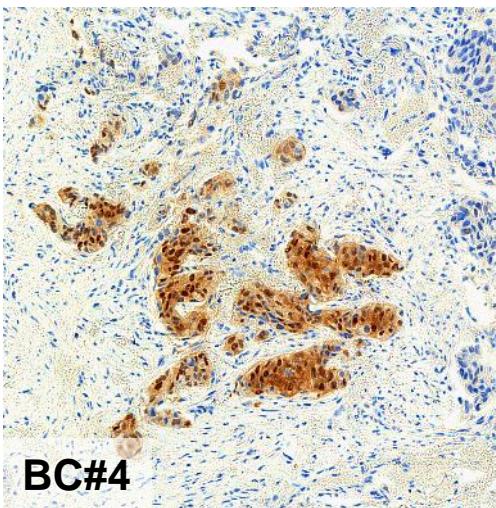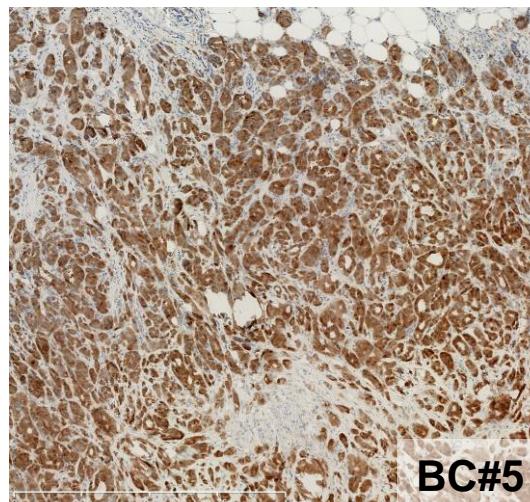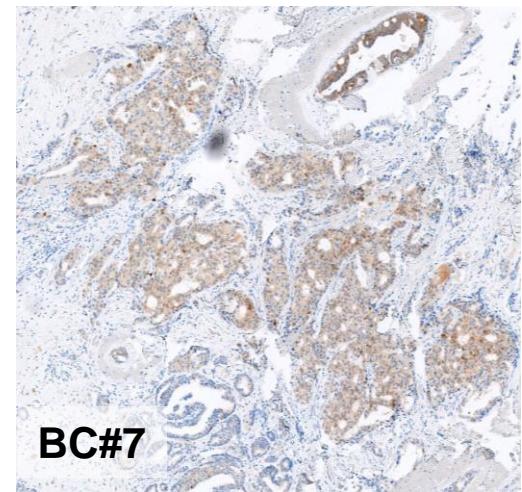
NY-BR-1-specific CD4$^+$ T cells are among PBMCs obtained from breast cancer patients (BC)
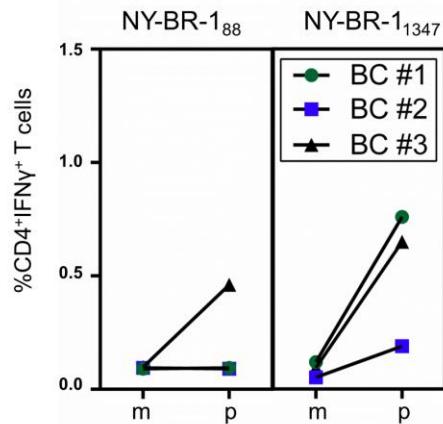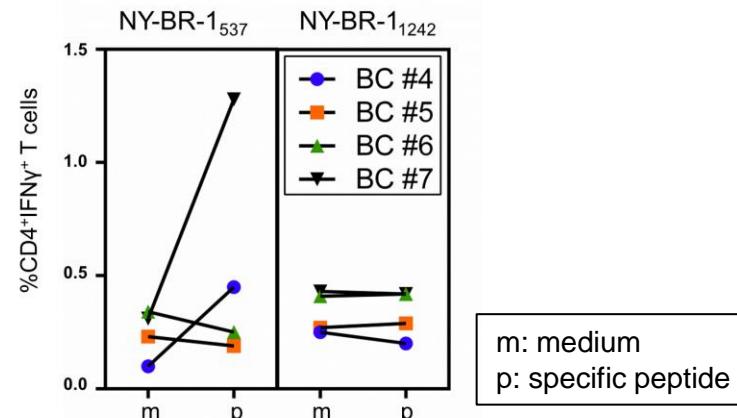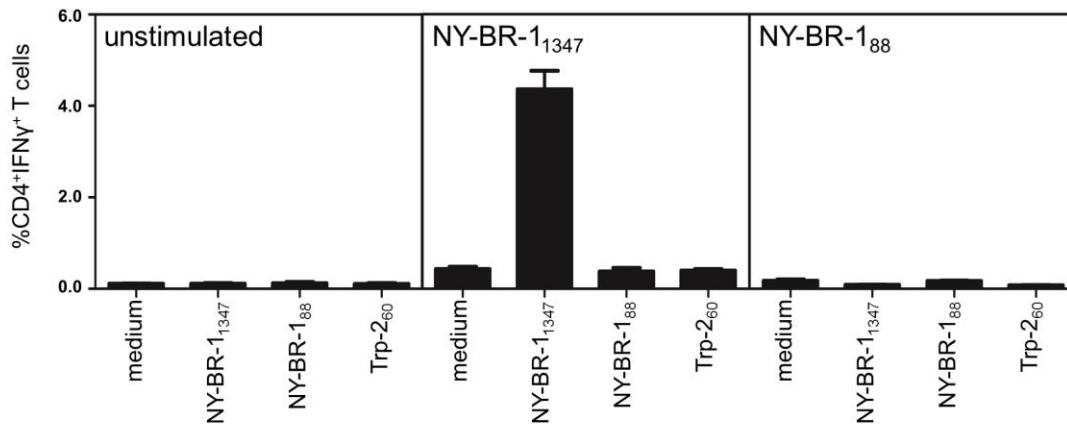
patients have specific T cells ✓

Summary

1. Identification of NY-BR-1-specific, HLA-restricted CD4$^+$ T cell epitopes

2. Establishment of murine HLA-restricted CD4$^+$ T cell lines

3. Confirmation of endogenous processing of the newly identified epitopes in human cells

4. Detection of NY-BR-1-specific CD4$^+$ T cells among PBMCs of breast cancer patients

Outlook

1. Detection of NY-BR-1-specific CD4$^+$ T cells among PBMCs of breast cancer patients (ex vivo), using **NY-BR-1-specific, HLA-DRB1*0301-/HLA-DRB1*0401-restricted MHC-II tetramers**

   => Immunomonitoring of specific CD4+ cells

2. Sequencing and cloning of a **NY-BR-1-specific TCR**

   => Usage of epitopes as helper peptides for vaccine development

DKFZ (Heidelberg)

Prof. Stefan Eichmüller
Dr. Wolfram Osen
Dr. Adriane Gardyan
Krishna Das
Elke Dickes

**Thank you
for your attention!**

Cooperations

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University Hospital Heidelberg)

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(National Center of Tumor
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50 Years – Research for
A Life Without Cancer

Backup

HLA-transgenic mouse models

DR3tg mice	DR4tg mice

genotype

MHC-I   mouse K^b/D^b mouse K^b/D^b

MHC-II   HLA-DRB1*0301/ $IA^{b-/-}$ HLA-DRB1*0401/ $IA^{b-/-}$

Screening of a NY-BR-1-specific library leads to the identification of NY-BR-1 candidate epitopes

NY-BR-1 peptide library (combinatorial analysis)

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13
L1	1	14	27	40	53	66	79	92	105	118	131	144	157
L2	158	2	15	28	41	54	67	80	93	106	119	132	145
L3	174	159	3	16	29	42	55	68	81	94	107	120	133
L4	134	147	160	4	17	30	43	56	69	82	95	108	121
L5	122	135	148	161	5	18	31	44	57	70	83	96	109
L6	110	123	136	149	162	6	19	32	45	58	71	84	97
L7	98	111	124	137	150	163	7	20	33	46	59	72	85
L8	86	99	112	125	138	151	164	8	21	34	47	60	73
L9	74	87	100	113	126	139	152	165	9	22	35	48	61
L10	62	75	88	101	114	127	140	153	170	10	23	36	49
L11	50	63	76	89	102	115	128	141	154	167	11	172	37
L12	38	51	64	77	90	103	116	129	142	155	168	12	25
L13	26	39	52	65	78	91	104	117	130	143	156	169	13

Epitope containing library peptides recognized by spleen cells of **DR3tg** mice or **DR4tg mice** , respectively.

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