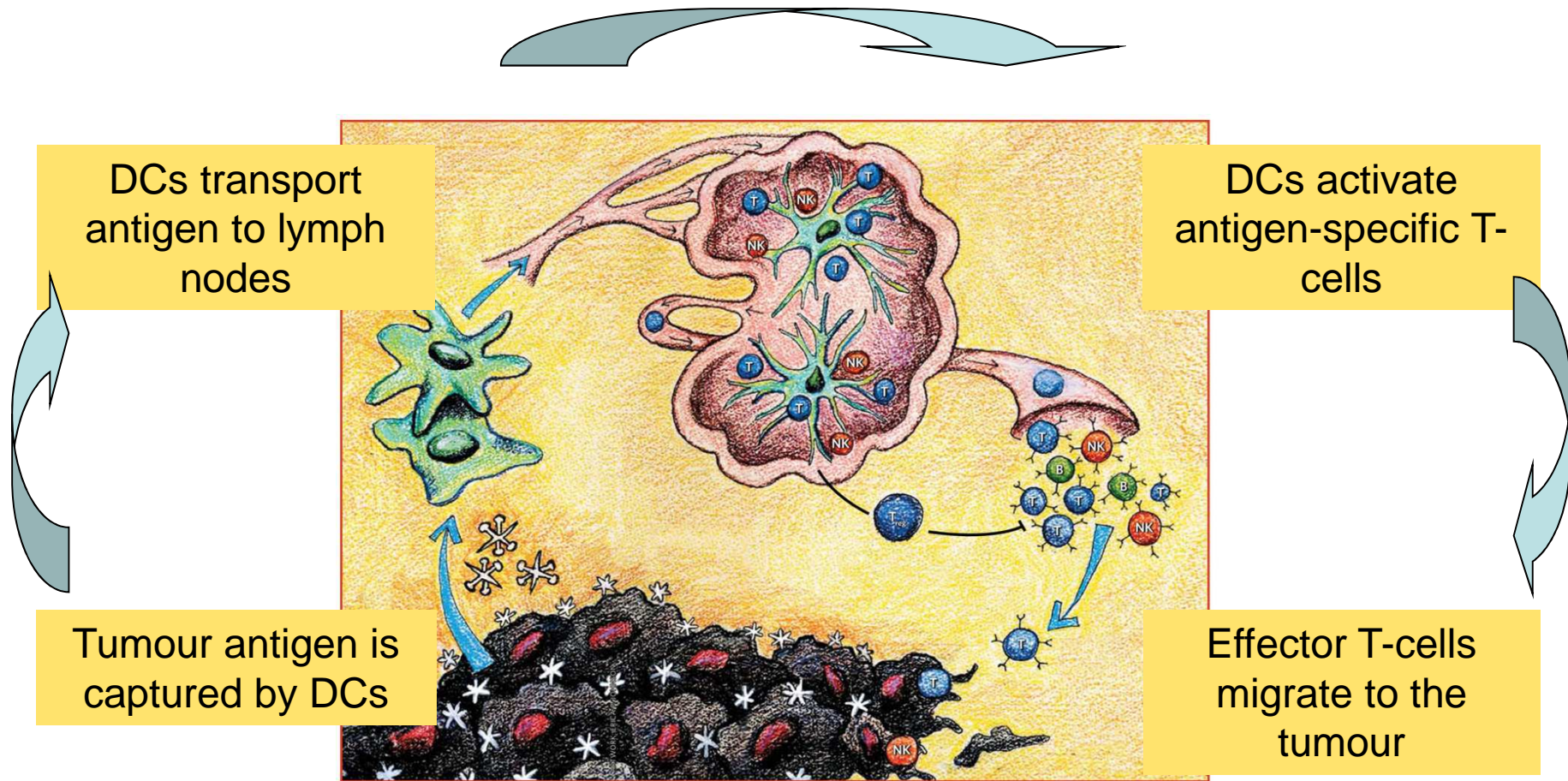


Cancer Immunotherapy

Henning Walczak

Basic features of a 'spontaneous' anti-tumour T-cell response





Case History:

Previously healthy woman, 53 years old

03-03-97: awoke dizzy

- severe vertigo
- unintelligible speech
- truncal and appendicular ataxia

07-03-97: unable to sit, stand, use hands

Correct diagnosis:

Breast Cancer

Paraneoplastic cerebellar degeneration (PCD)

Case History:

Previously healthy woman, 53 years old

03-03-97: awoke dizzy

- severe vertigo
- unintelligible speech
- truncal and appendicular ataxia

07-03-97: unable to sit, stand, use hands

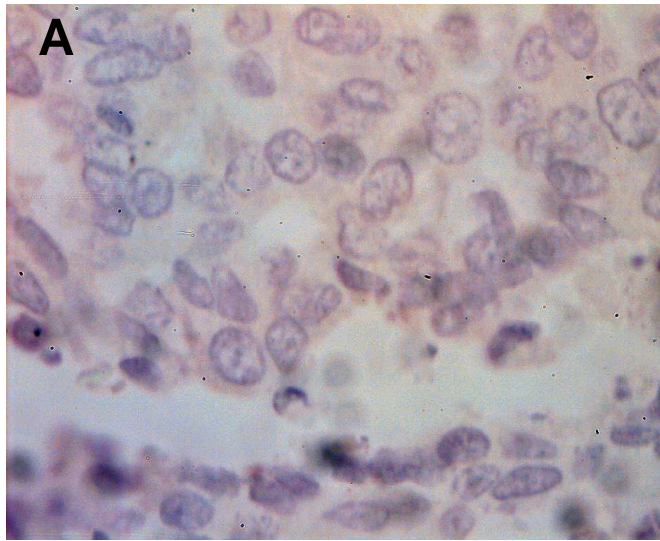
21-03-97: detection of anti-**CDR2** antibody in the serum

27-03-97: detection of occult breast cancer

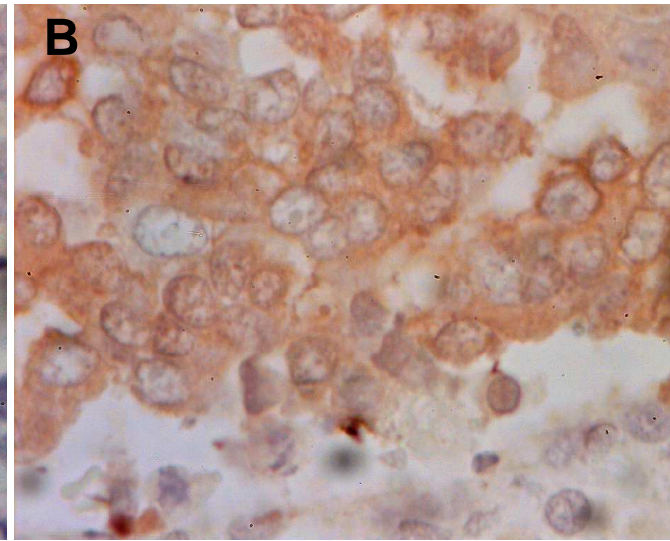
CDR2 = cerebellum degeneration-related antigen 2

PCD patient serum reacts with CDR2 protein in tumour tissue

Sections of breast tumour

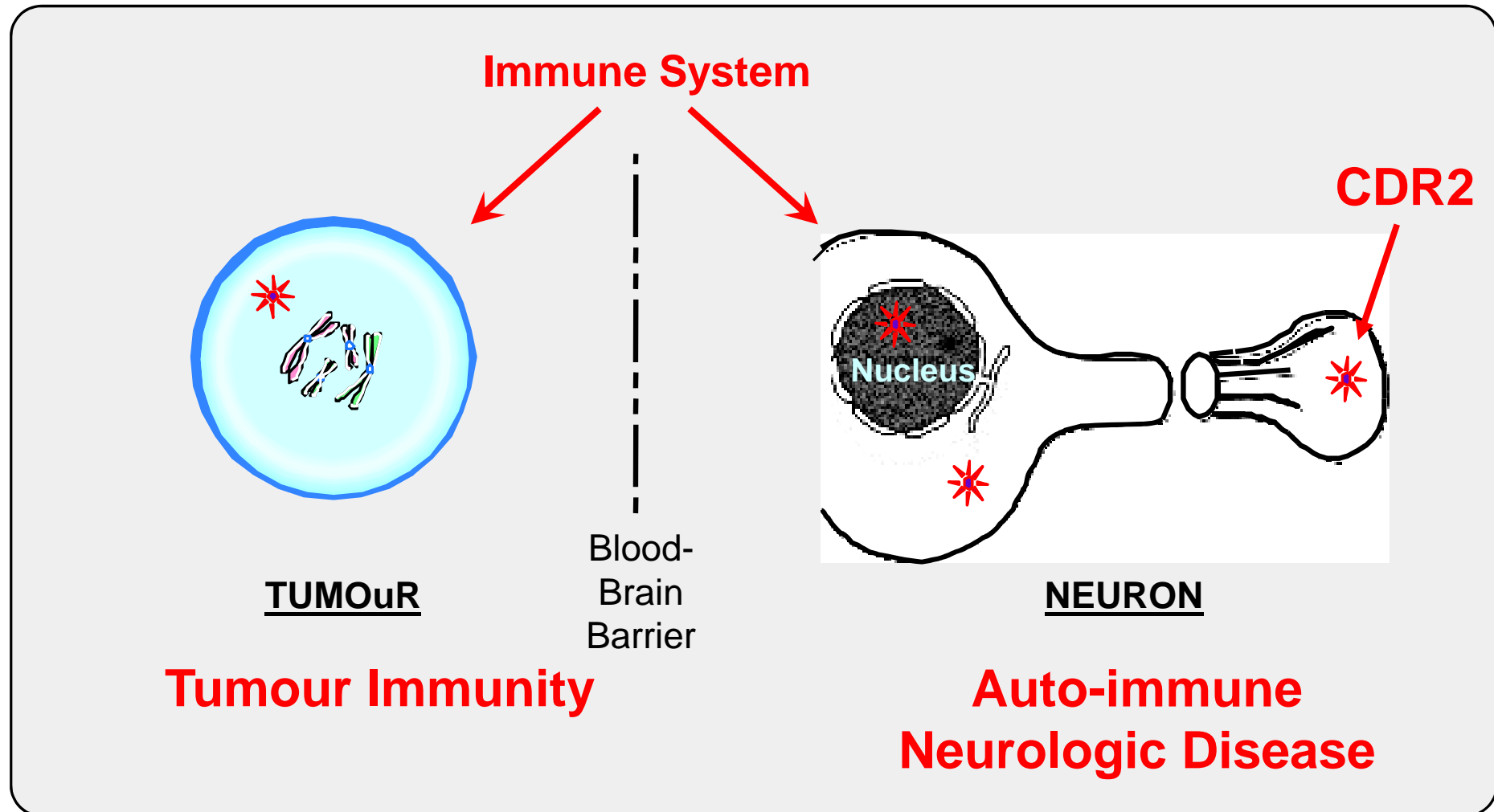


Control serum



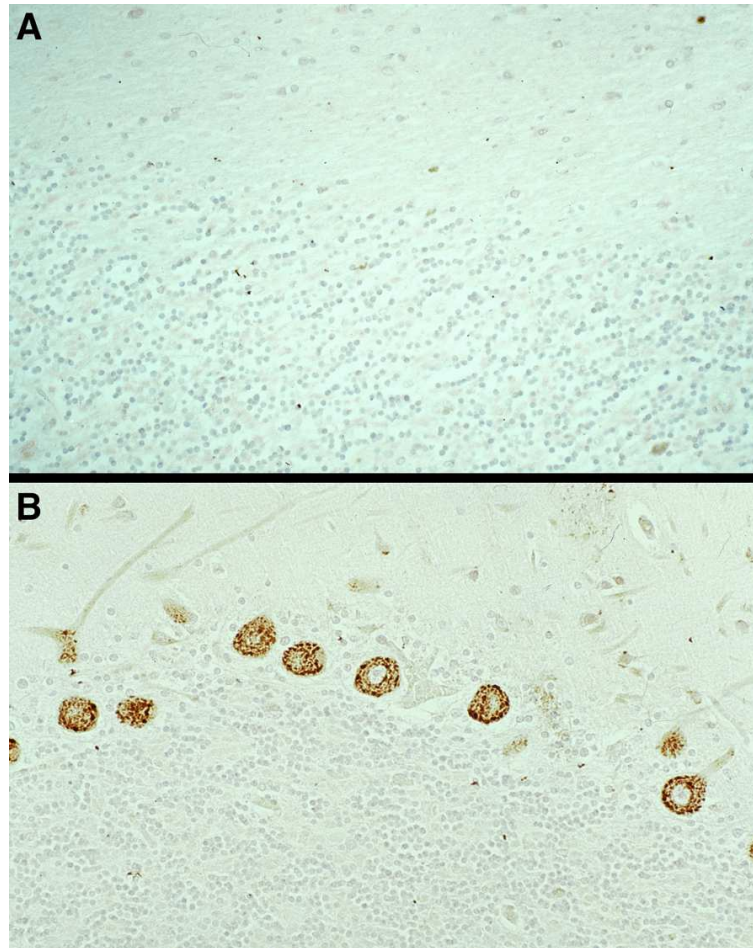
PCD patient serum

Spontaneous immunity against tumour-expressed antigen results in auto-immune disease

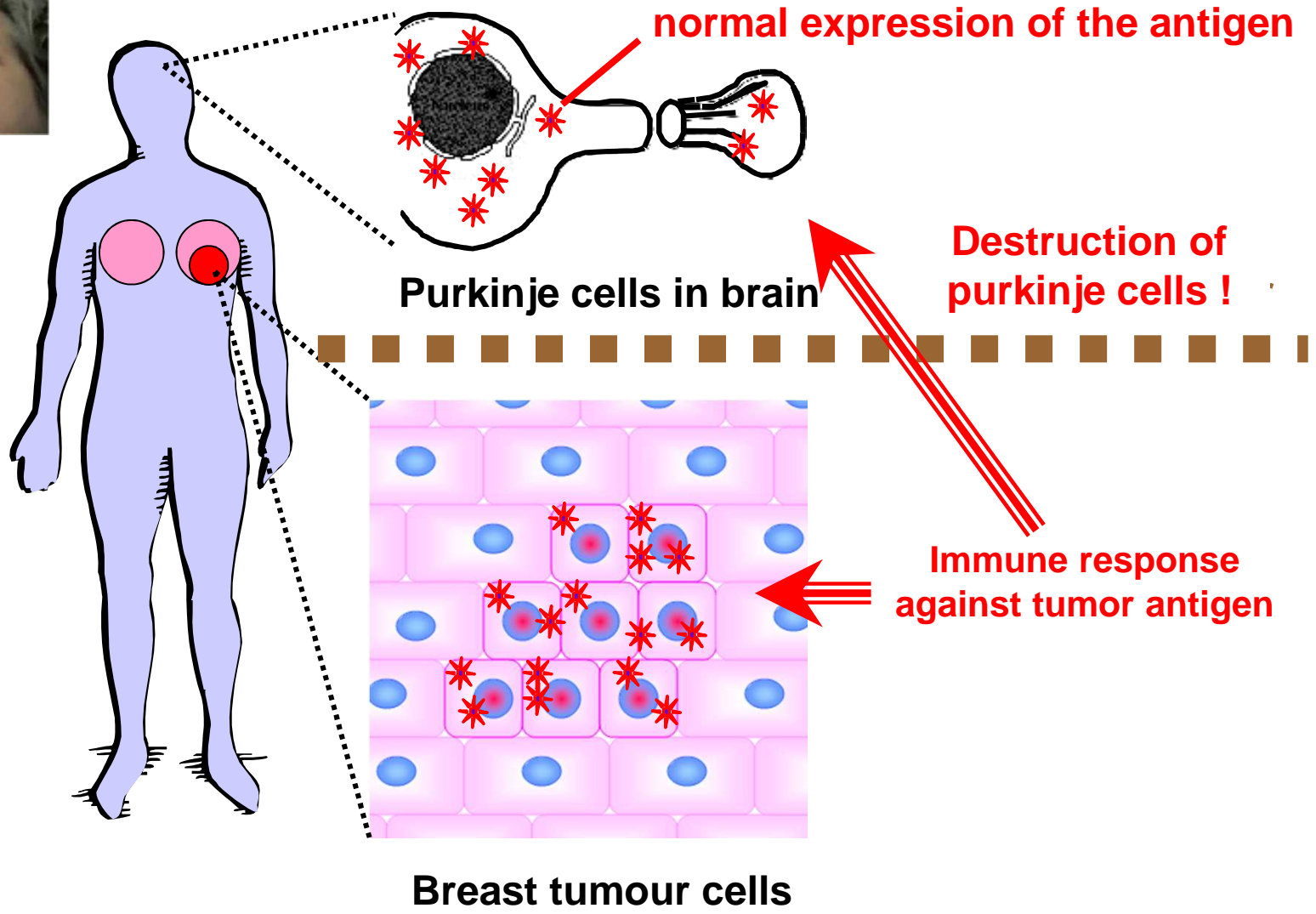


Elimination of Purkinje cells by tumour-induced auto-immune response causes PCD

**PCD
Brain**



Control Brain



What does this example teach us?

- 1. At least certain tumours can express antigens that are absent from corresponding normal tissues**
- 2. The immune system can, in principle, detect such abnormally expressed antigens and, as a result, launch an effective attack against the tumour**
- 3. In certain cases, this may result in auto-immune destruction of normal somatic tissues**

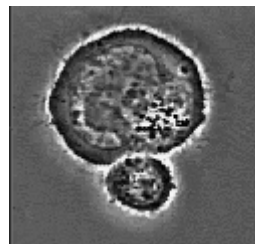
Tumour Immunology

1

Study of the interaction between tumour and immune system

2

Development of **safe** immunotherapeutic strategies against cancer



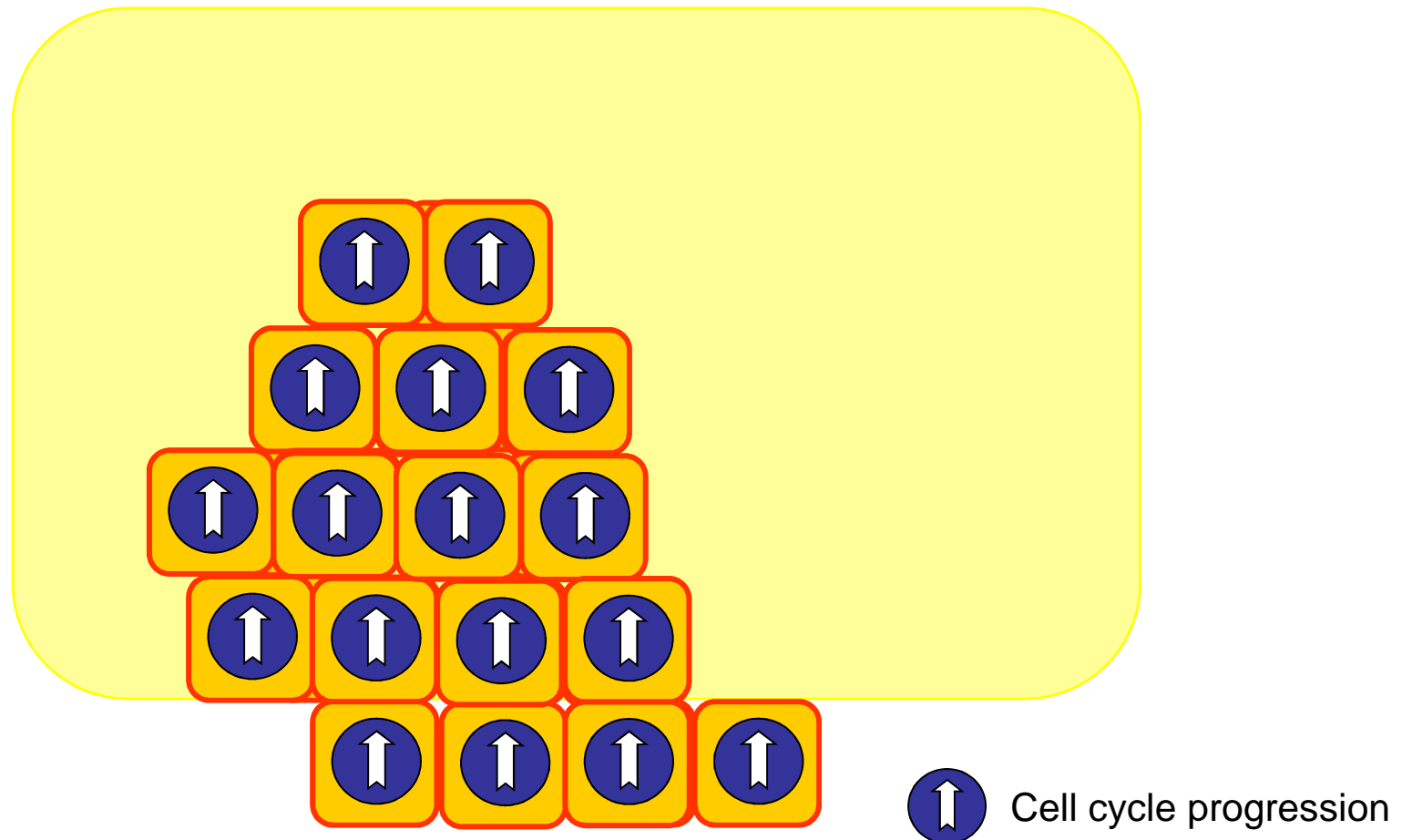
Initiation of cancer usually results from (a) sporadic event(s)

- Irradiation
- Chemical mutagens
- Spontaneous errors during DNA replication
- Tumor virus-induced changes in genome

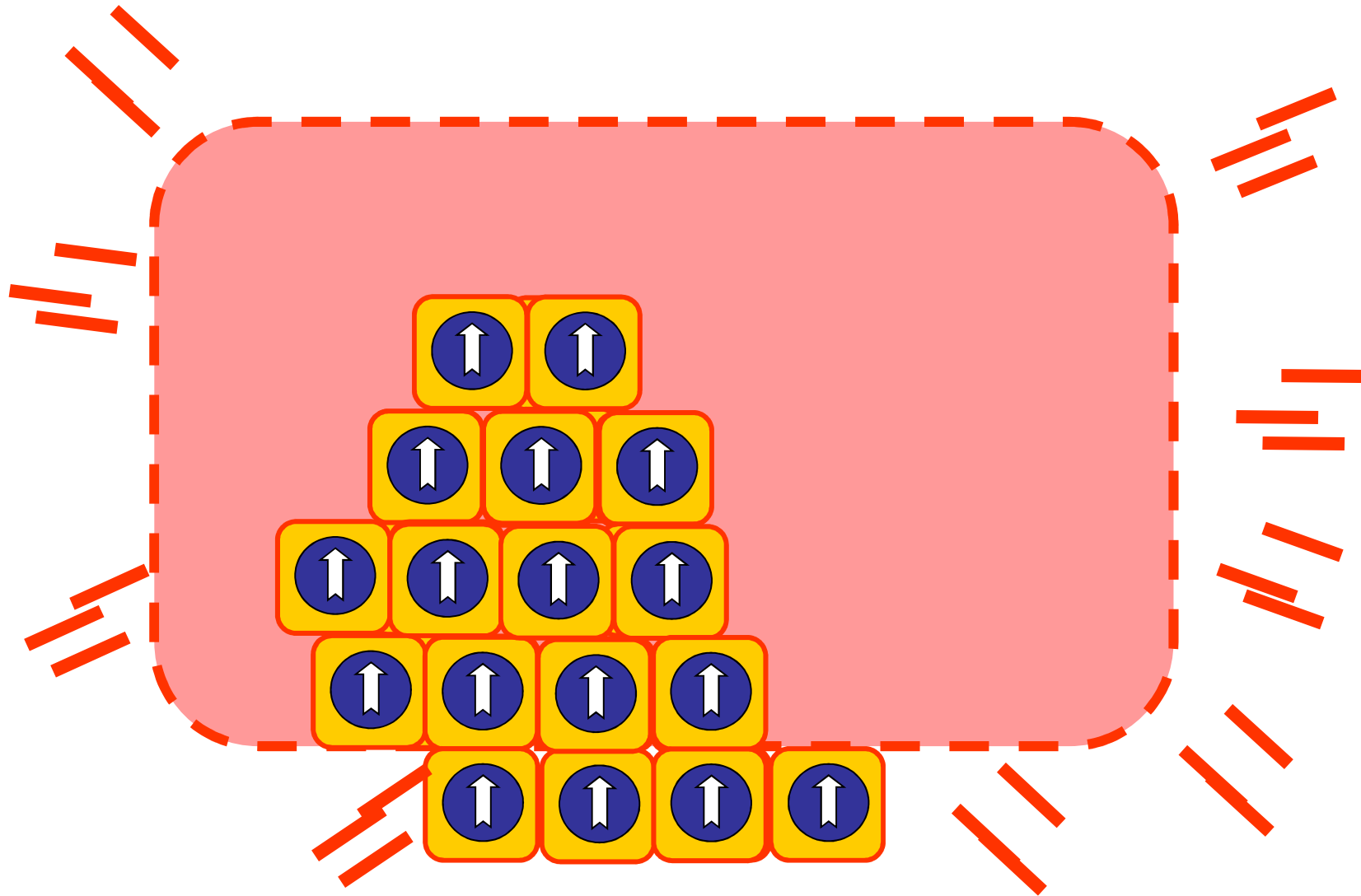


Induction of mutations in cellular DNA

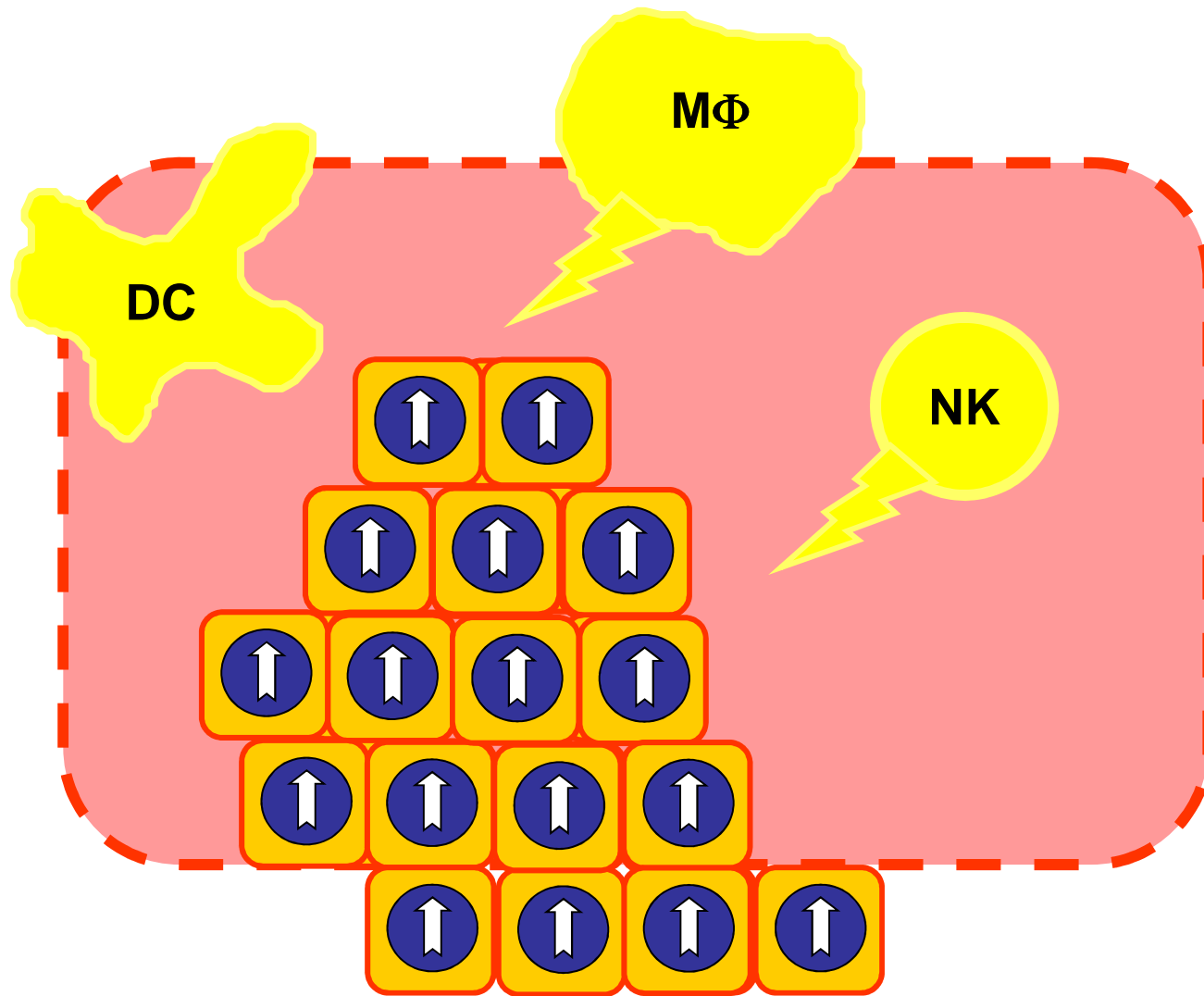
Absence of apoptosis and of cell cycle regulation results in tumour growth



Tumor growth (eventually) results in inflammatory signals

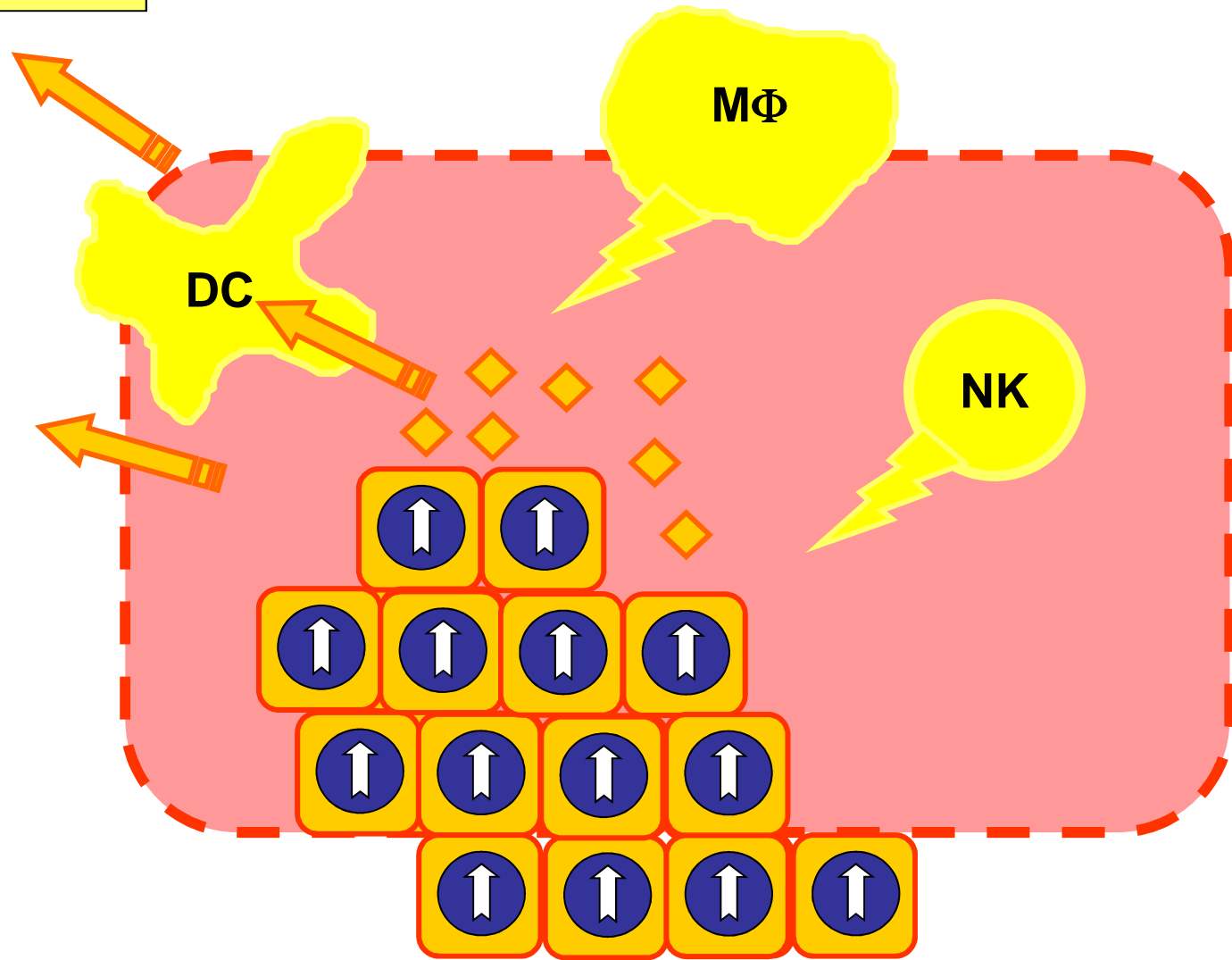


Recruitment of innate immunity

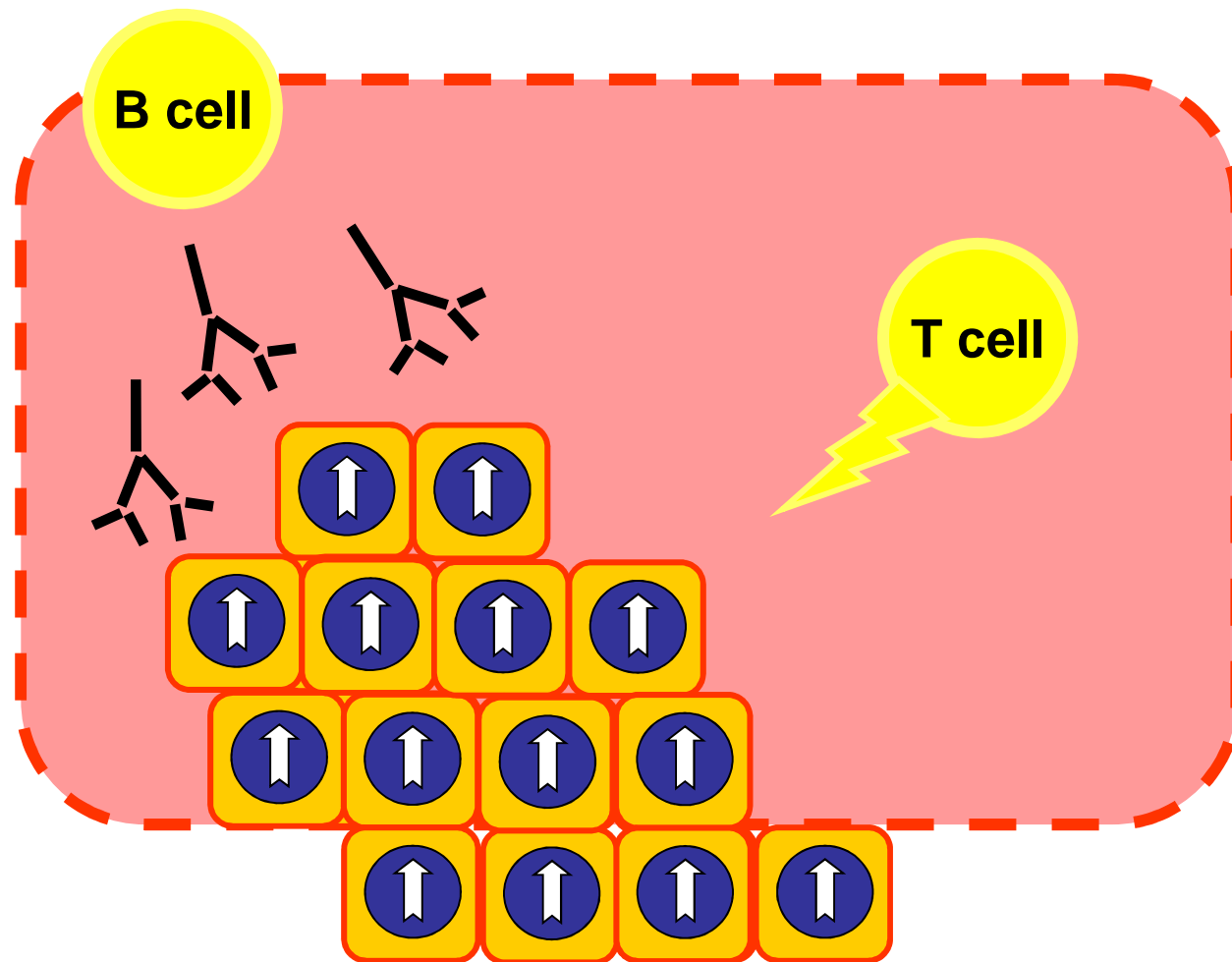


Recruitment of innate immunity

Draining lymphnode



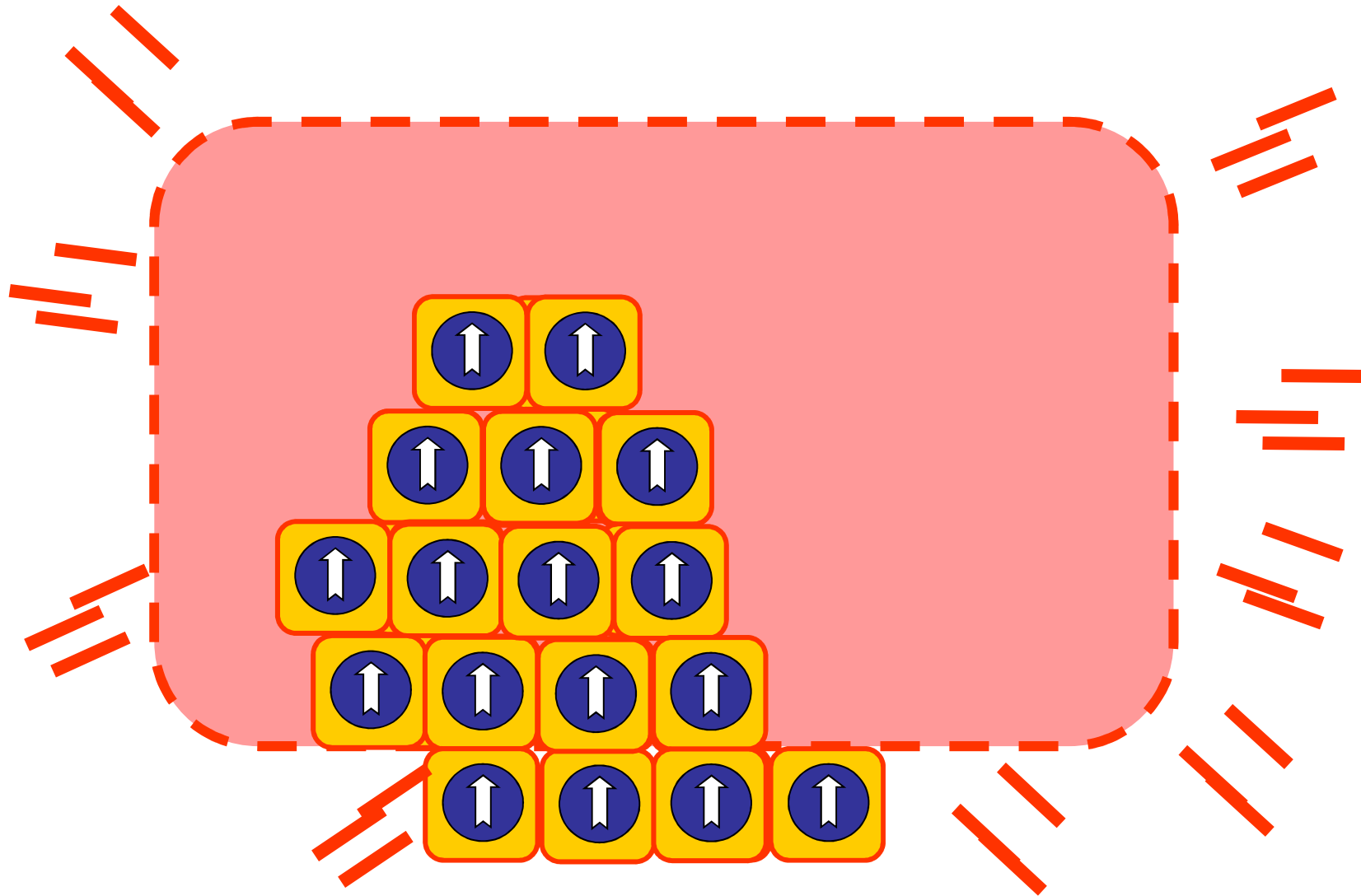
Recruitment of adaptive, antigen-specific immunity



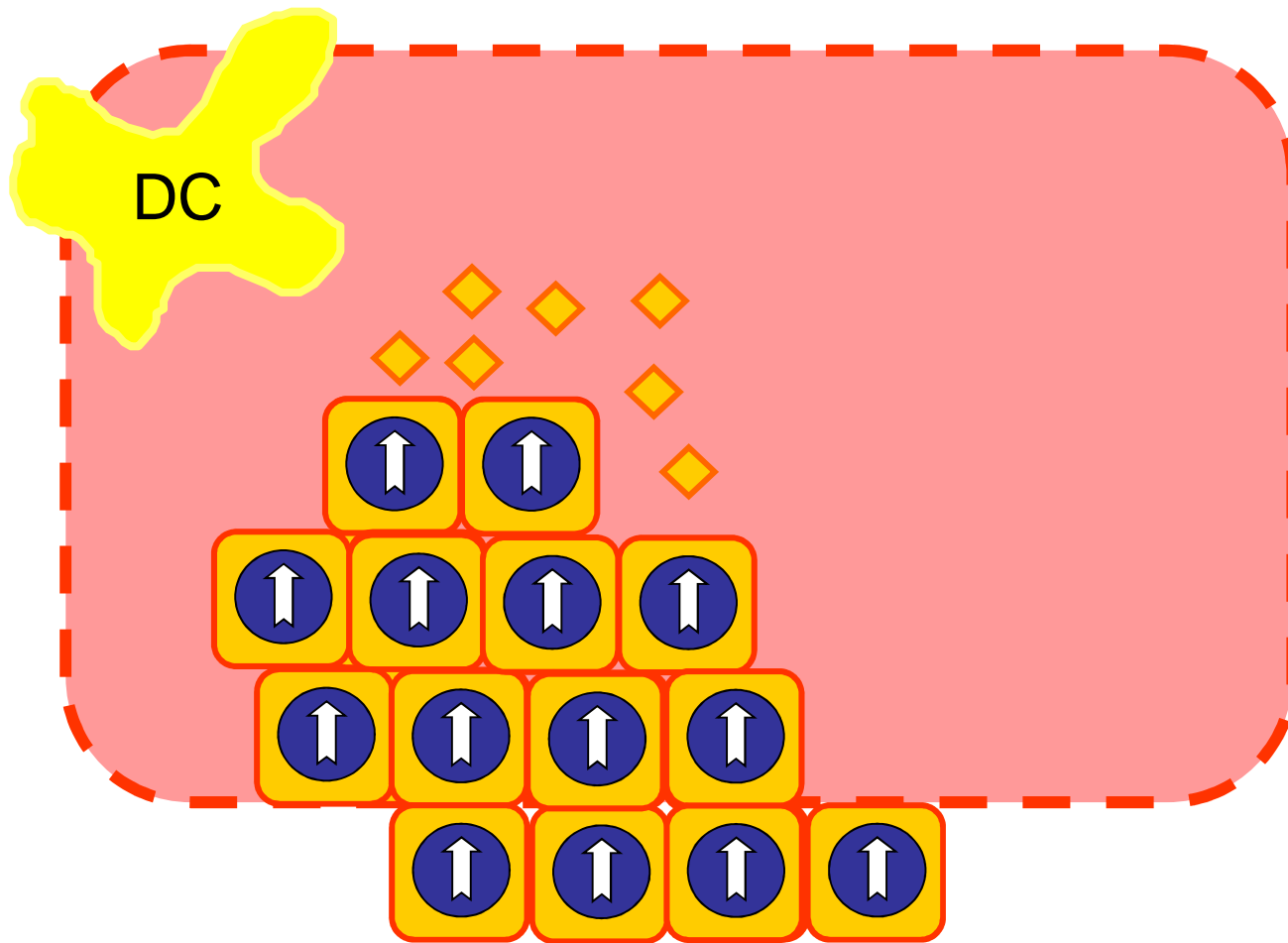
Requirements for activation of an adaptive anti-tumour immune response

- 1. Local inflammation in the tumour**
- 2. Expression and recognition of
tumour antigens**

Problems in immune surveillance of cancer: #1
It takes the tumour a while to cause local inflammation



Problems in immune surveillance of cancer: #2
Antigenic differences between normal and tumour cells
can be very subtle



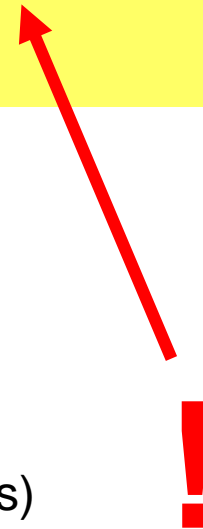
If requirements for 'spontaneous' activation of the adaptive anti-tumour immune response were not met, could we **teach** the immune system to **selectively** detect and destroy tumour cells?



Cancer Immunotherapy

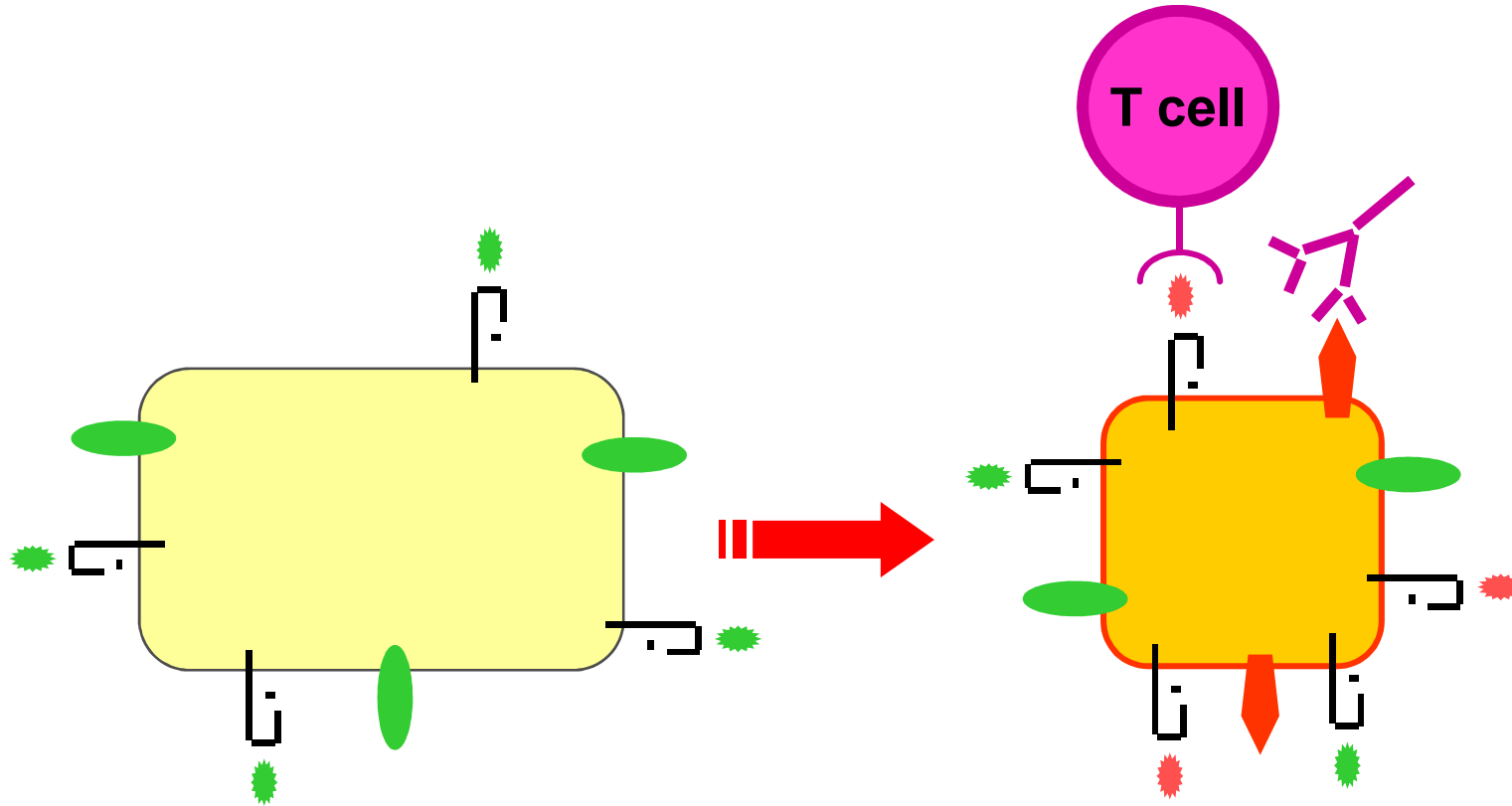
(A possible alternative to conventional therapies)

Which antigens should be targeted?



1

Tumour-specific antigens



1

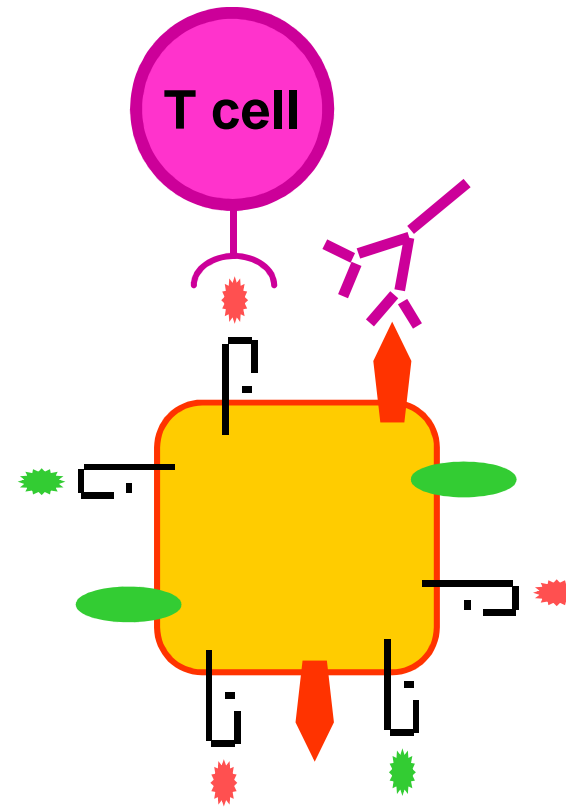
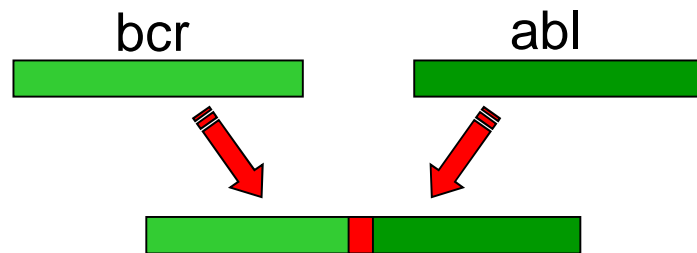
Tumour-specific antigens

- Viral proteins
 - Epstein Barr Virus (EBV)
 - Human Papillomavirus (HPV)

- Mutated cellular proteins
 - TGF- β receptor III



- Bcr-Abl



Cancers with viral origin

Opportunistic malignancies:

- EBV-positive lymphoma
- HHV8-positive Kaposi sarcoma

Also in immunocompetent individuals:

- HTLV1-associated lymphoma
- HBV- and HCV-associated hepatocellular carcinoma
- HPV-positive genital cancers

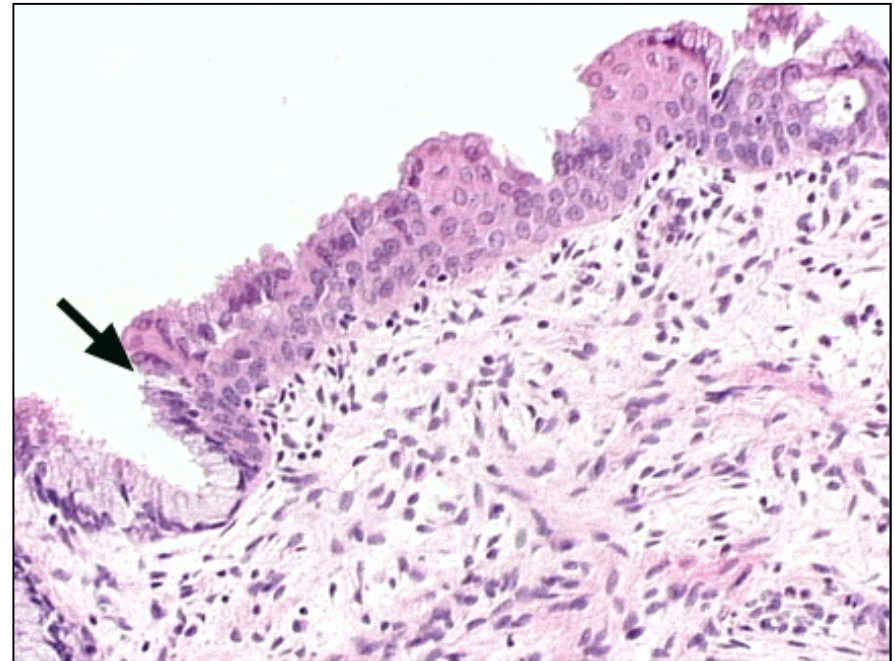
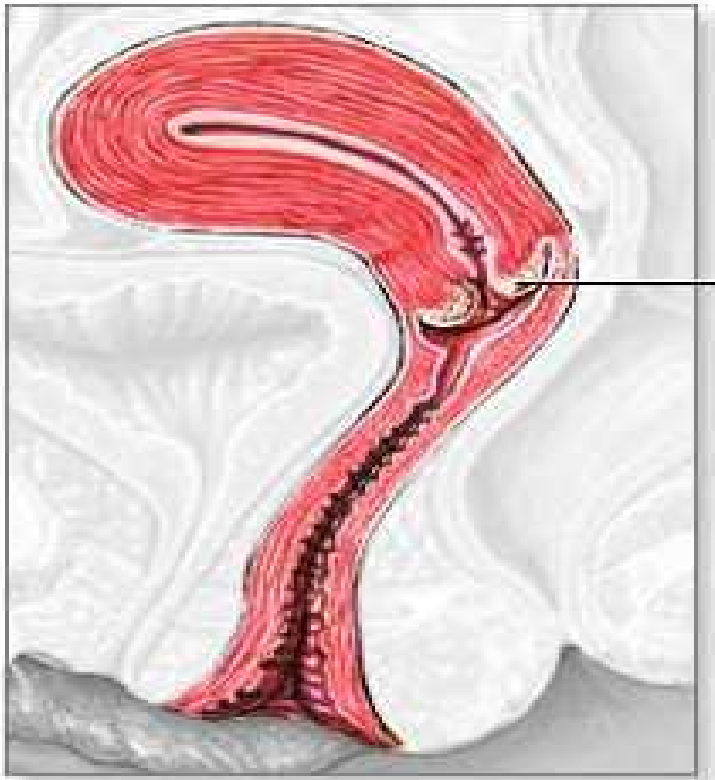


Direct relation between sexual behaviour and risk for HPV infection (study in college women)

Ho et al. (1998) N Engl J Med 338, 423

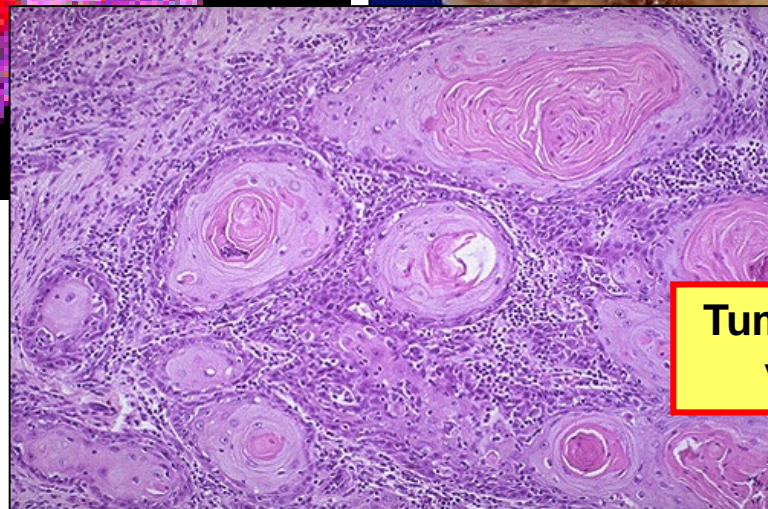
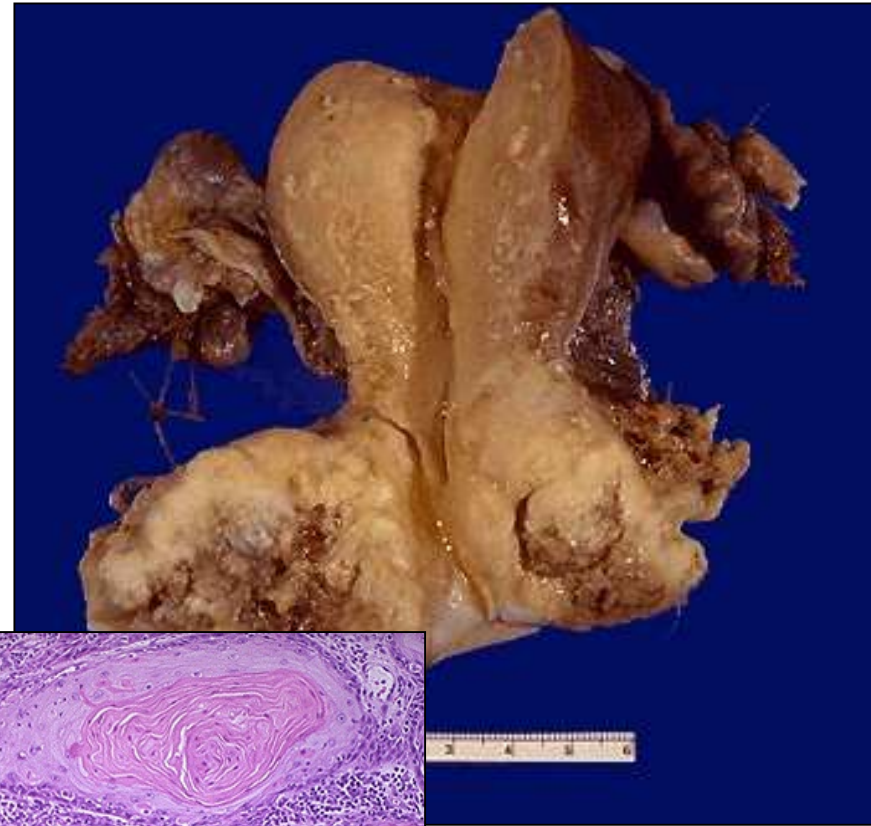
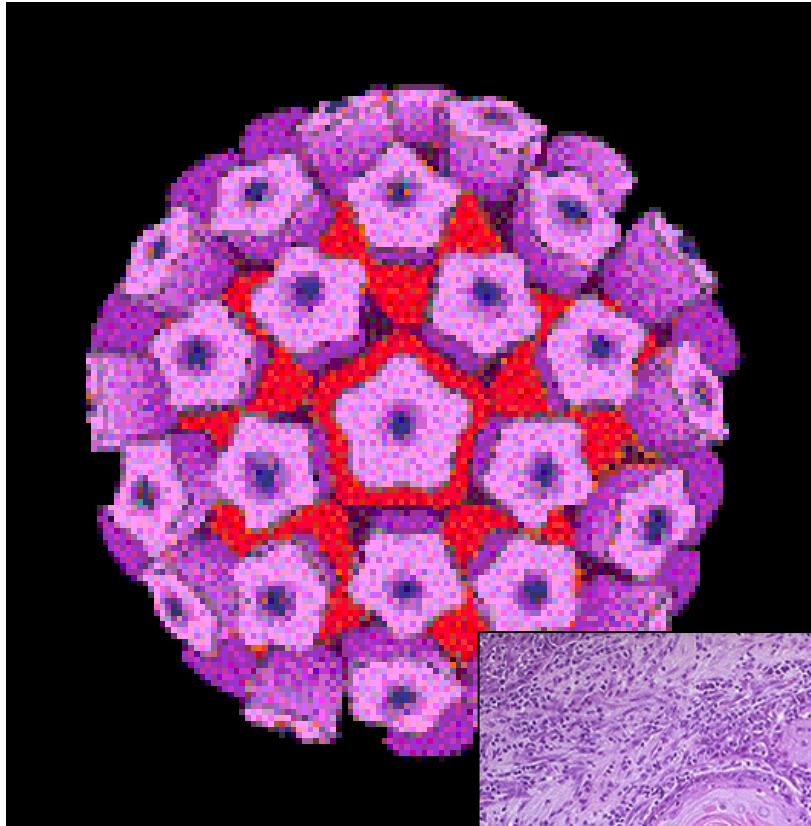
No. of male vaginal sex partners in previous 6 months	Adjusted relative risk	P value
0-3	1.0	< 0.001
4	3.6	
No. of male vaginal sex partners in previous 7-12 months		
0	1.0	< 0.001
1	1.7	
2-3	3.0	
≥ 4	4.2	
No. of lifetime sex partners of regular partners		
1	1.0	< 0.001
2-5	5.8	
≥ 6	10.1	

Cervical neoplasia starts with genital HPV infection



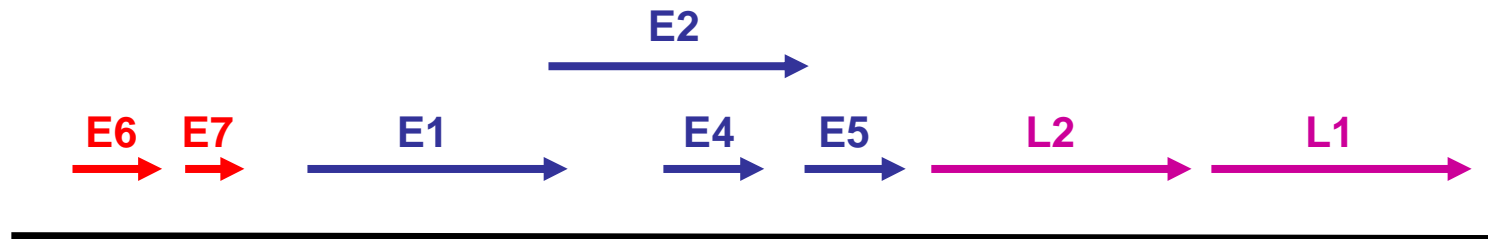
Human Papillomavirus (HPV)

Cervical Cancer

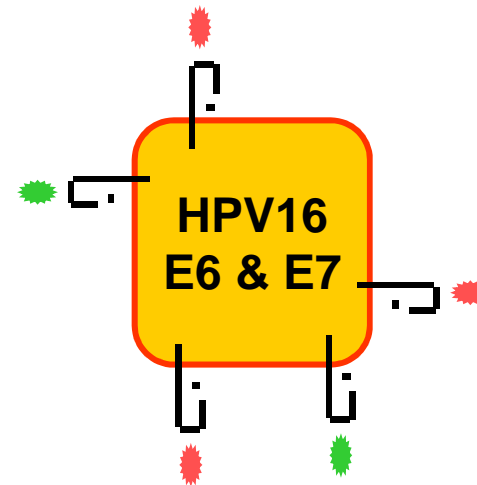


**Tumor cells express
viral antigens!**

Cervical cancer is induced and maintained by the **E6 and E7 oncoproteins of HPV**

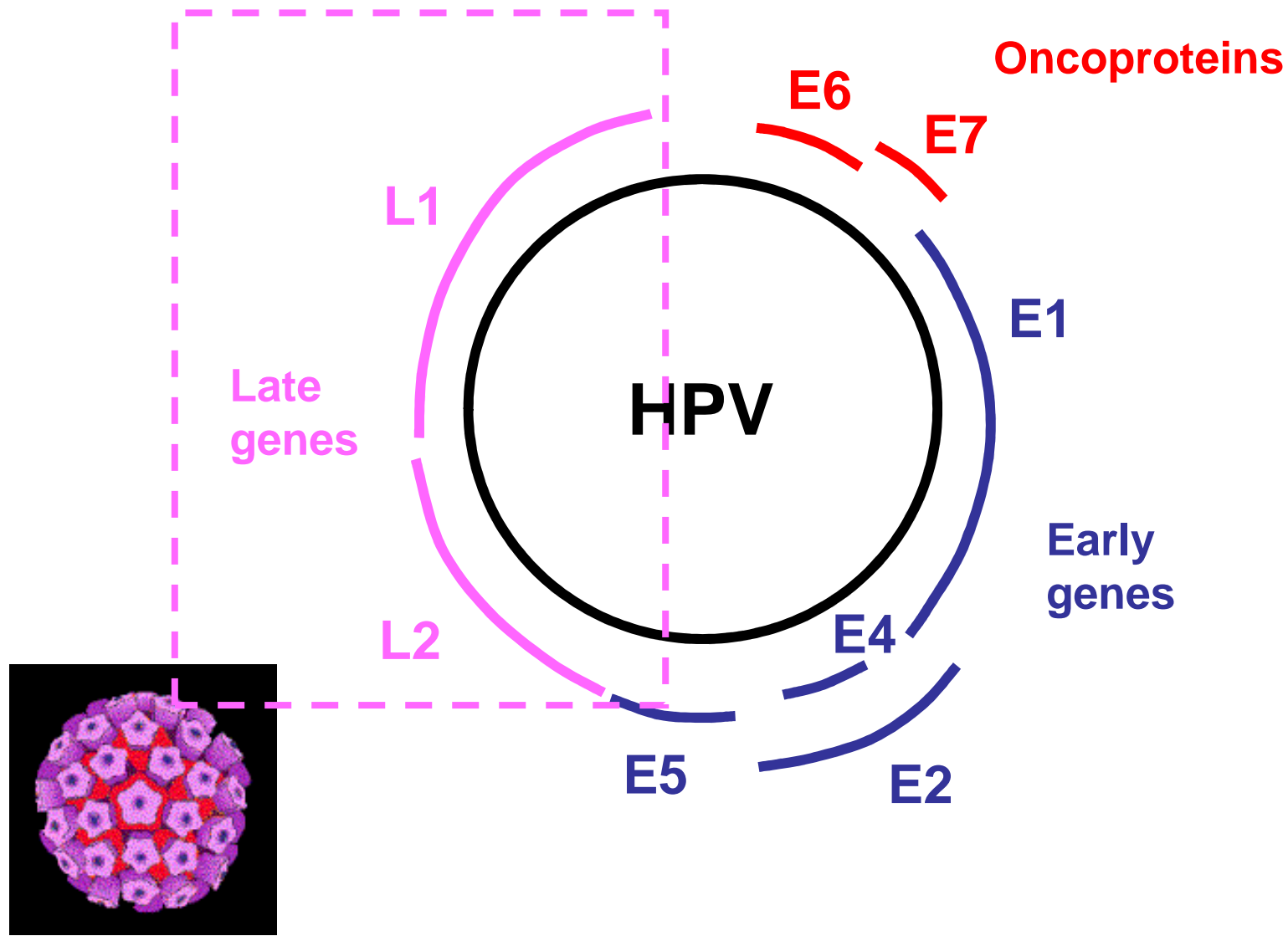


E6 and E7 are
intracellular
antigens



Nobel Prize for Physiology and Medicine 2008, Harald Zur Hausen

Target antigens for preventive HPV vaccination



The New England Journal of Medicine

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VOLUME 347

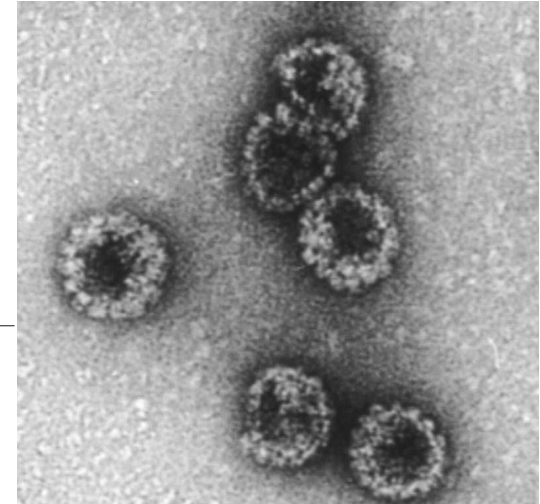
NOVEMBER 21, 2002

NUMBER 21



A CONTROLLED TRIAL OF A HUMAN PAPILLOMAVIRUS TYPE 16 VACCINE


LAURA A. KOUTSKY, PH.D., KEVIN A. AULT, M.D., COSETTE M. WHEELER, PH.D., DARRON R. BROWN, M.D.,
ELIAV BARR, M.D., FRANCES B. ALVAREZ, R.N., LISA M. CHIACCHIERINI, PH.D., AND KATHRIN U. JANSEN, PH.D.,
FOR THE PROOF OF PRINCIPLE STUDY INVESTIGATORS



	Impact of vaccination	placebo	VLP vaccine	efficacy
17 mnd	persistent infection	41	0	100%
	transient infection	68	6	91%
	<i>total</i>	765	768	
48 mnd*	persistent infection	92	0	100%
	CIN lesions	24	0	100%
	<i>total</i>	750	755	

(* Mao et al 2006 Obst Gynecol)

GARDASIL—the only cervical cancer vaccine
For girls and young women ages 9 to 26 years



**YOU COULD BECOME
1 LESS LIFE AFFECTED
BY CERVICAL CANCER.**

GARDASIL is the only vaccine that may help guard against diseases that are caused by human papillomavirus (HPV) Types 6, 11, 16, and 18:

- [Cervical cancer](#)
- Cervical abnormalities that can sometimes lead to [cervical cancer](#)
- [Genital warts](#)

HPV Types 16 and 18 cause 70% of cervical cancer cases, and HPV Types 6 and 11 cause 90% of genital warts cases.

IMPORTANT INFORMATION ABOUT GARDASIL

GARDASIL may not fully protect everyone and does not prevent all types of cervical cancer, so it is important to continue regular cervical cancer screenings.

Anyone who is allergic to the ingredients of GARDASIL should not receive the vaccine. GARDASIL is not for women who are pregnant.

GARDASIL will not treat these diseases and will not protect against diseases caused by other types of HPV.

GARDASIL is given as 3 injections over 6 months and can cause pain, swelling, itching, and redness at the injection site, fever, nausea, and dizziness. Only a doctor or healthcare professional can decide if GARDASIL is right for you or your daughter. Ask about GARDASIL today.

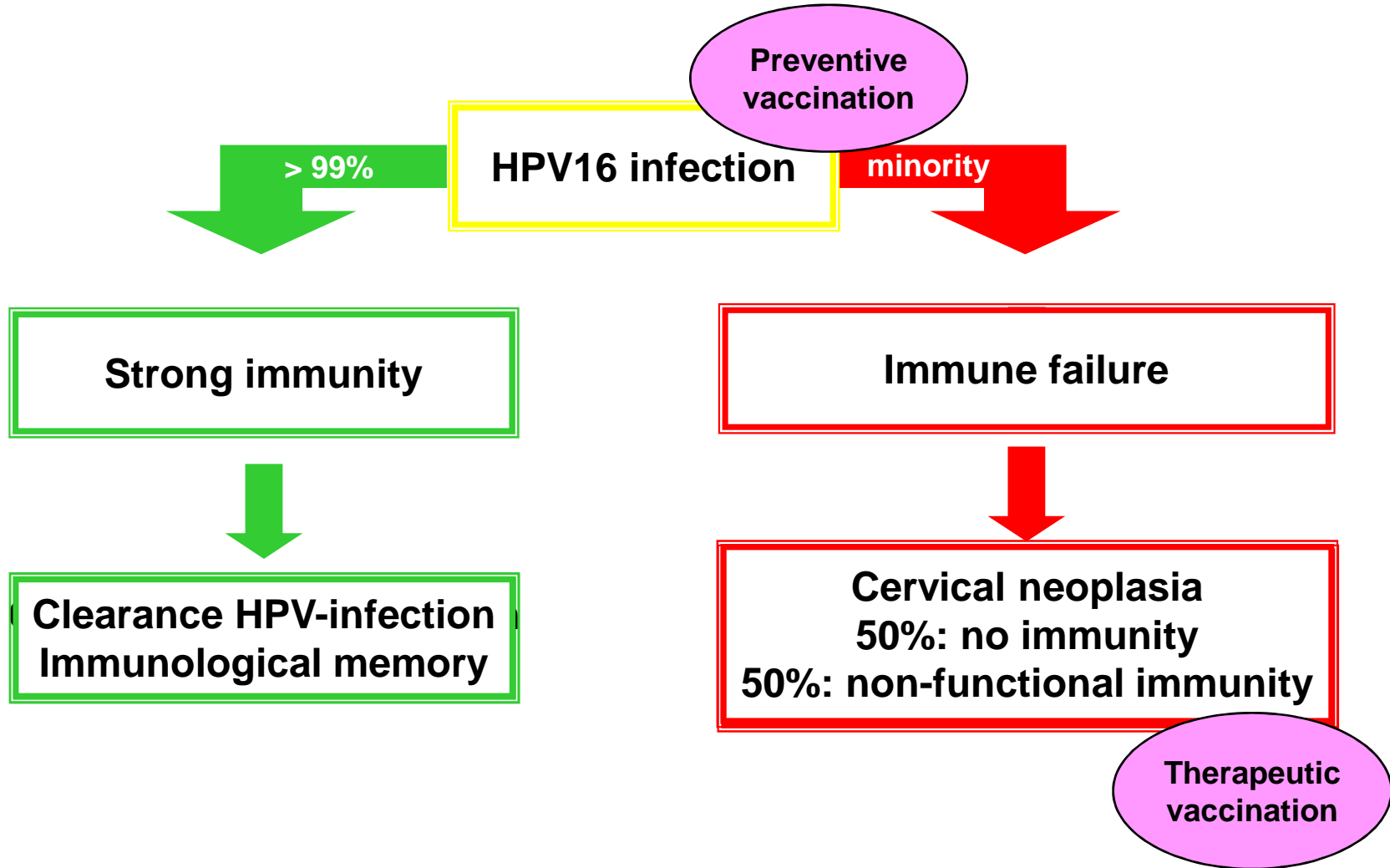
Now you can
DO SOMETHING

- » [Prepare to visit your doctor](#)
- » [Make sure you are covered](#)
- » [Request more information](#)

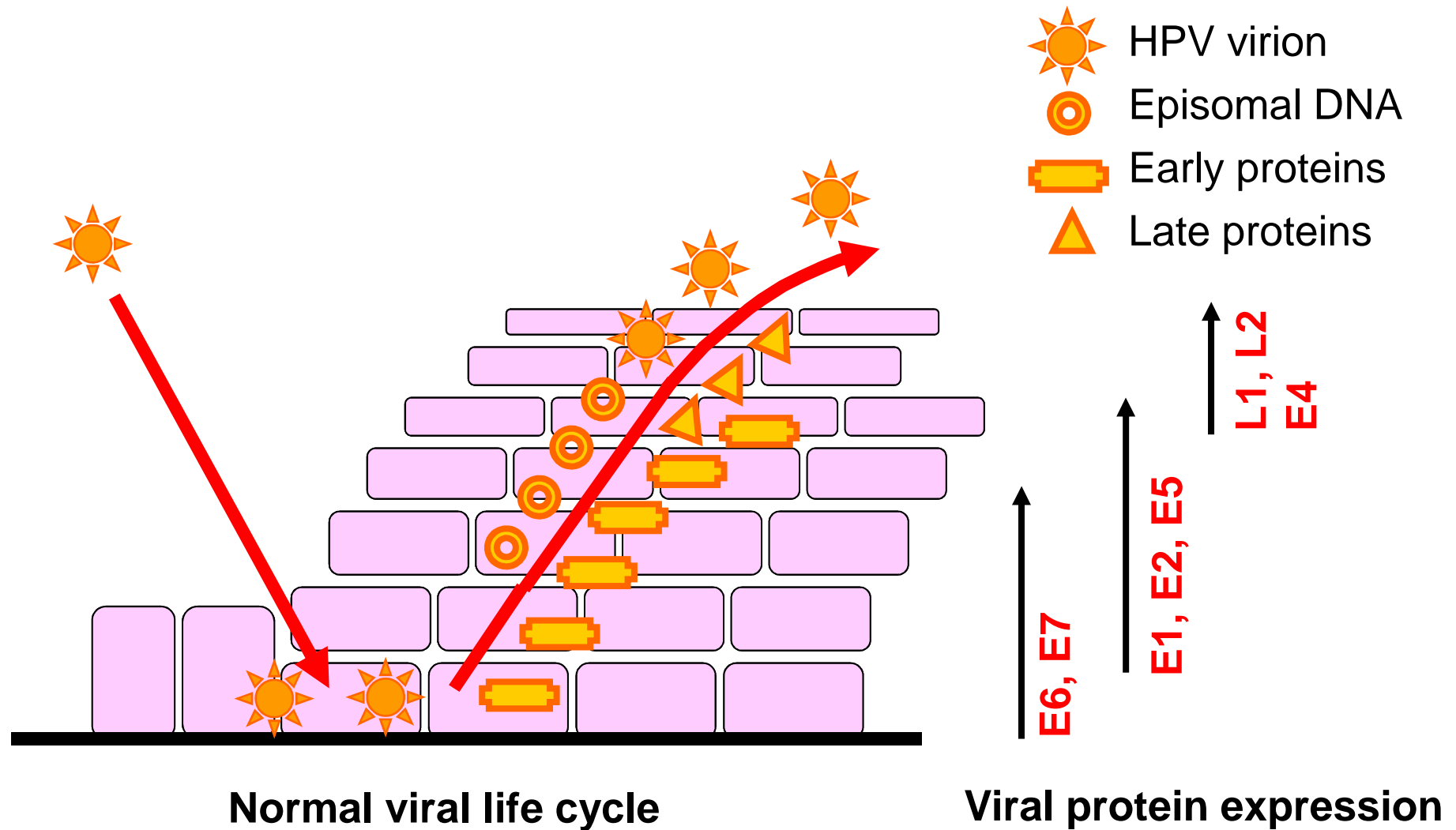


- » [See our TV ad](#)

Relation between consequences of cervical HPV infection and HPV-specific T cell immunity



The **infection cycle of HPV** is linked to the keratinocyte differentiation programme



Requirements for activation of an effective anti-viral immune response

1. Local inflammation
2. Target antigens

Question:

Which of these requirements is not met during cervical HPV infection?

Requirements for activation of an effective anti-viral immune response

1. Local inflammation
2. Target antigens

Largely lacking in genital HPV infection

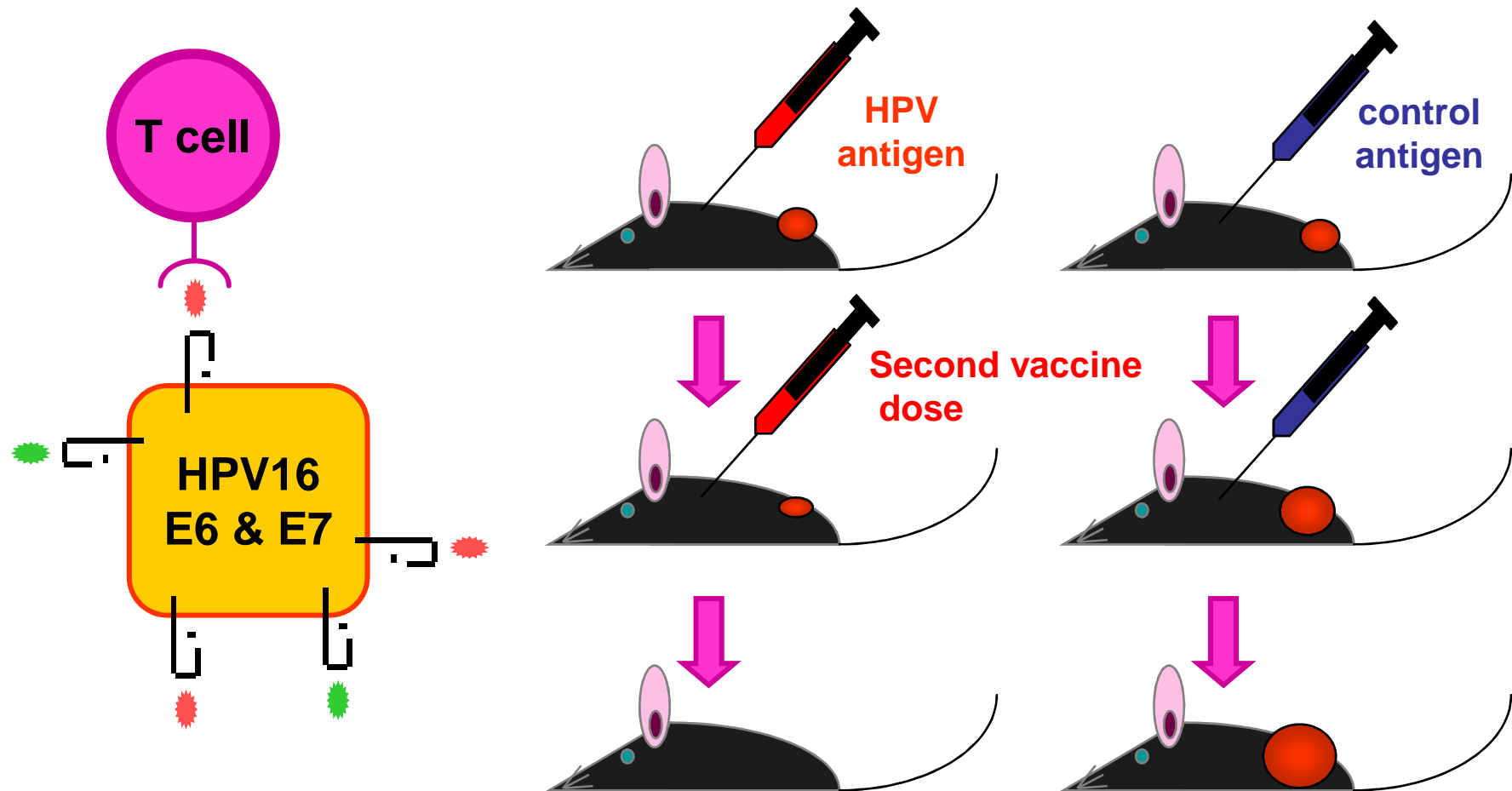
Consequences:

- Viral persistence for periods well over 1 year
- Neoplasia

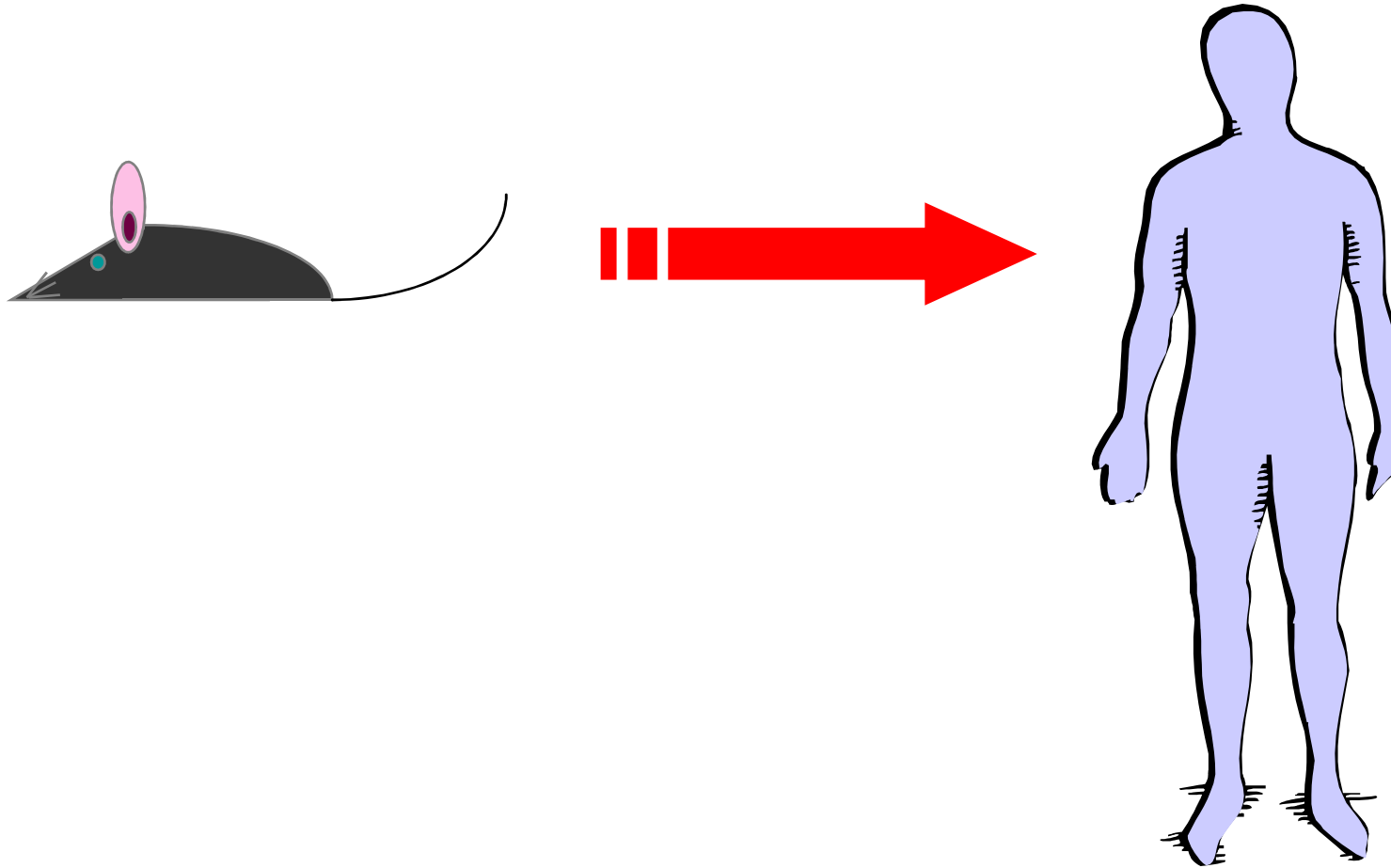
What is needed to trigger an effective HPV-specific immune response by **vaccination?**

- 1. Local inflammation (adjuvant)**
- 2. Tumour antigen expression and recognition**

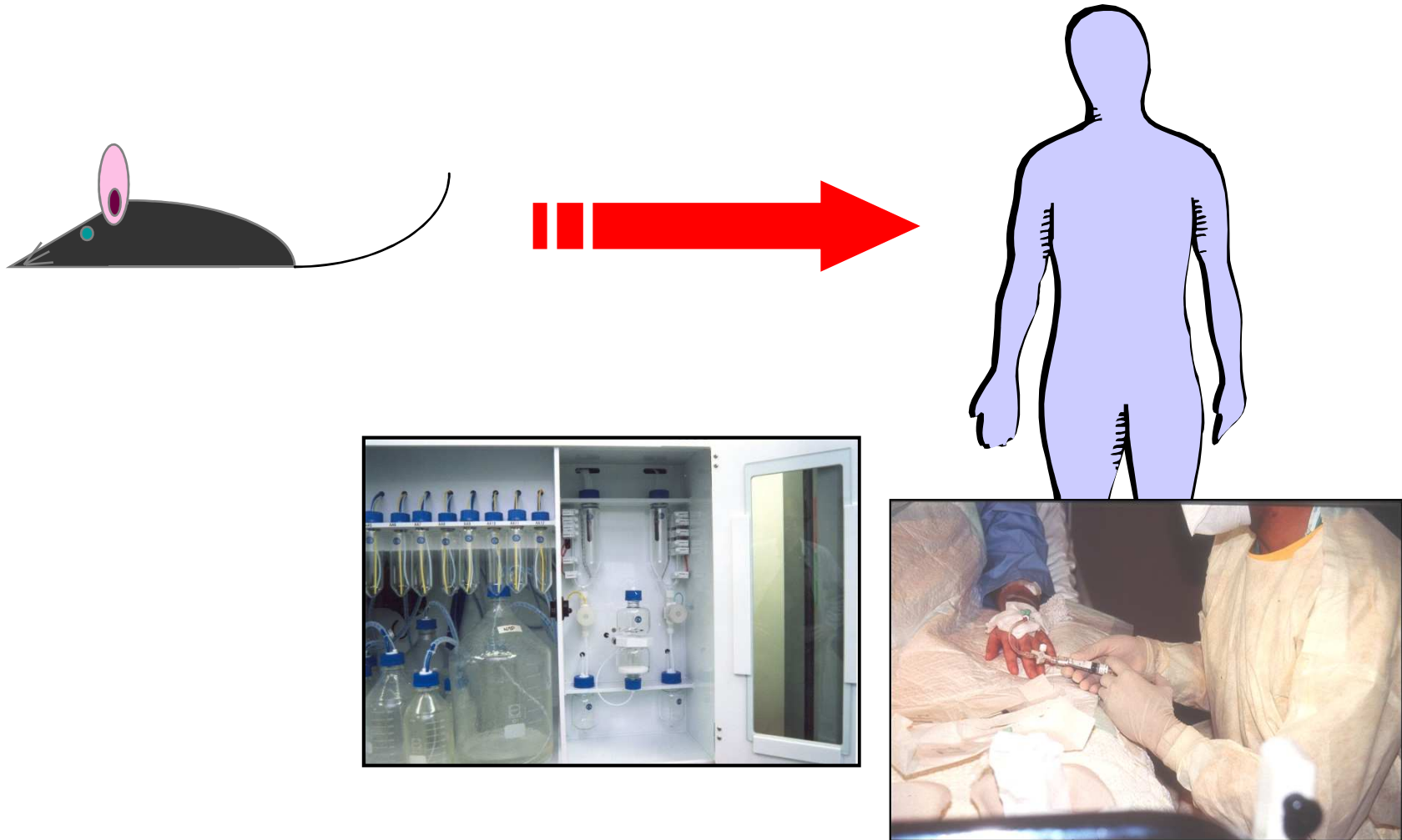
Therapeutic anti-tumour treatment in mice
by peptide vaccination
tumour antigen + adjuvants



Clinical testing of therapeutic peptide vaccine in patients with cervical cancer



Clinical testing of therapeutic peptide vaccine in patients with cervical cancer



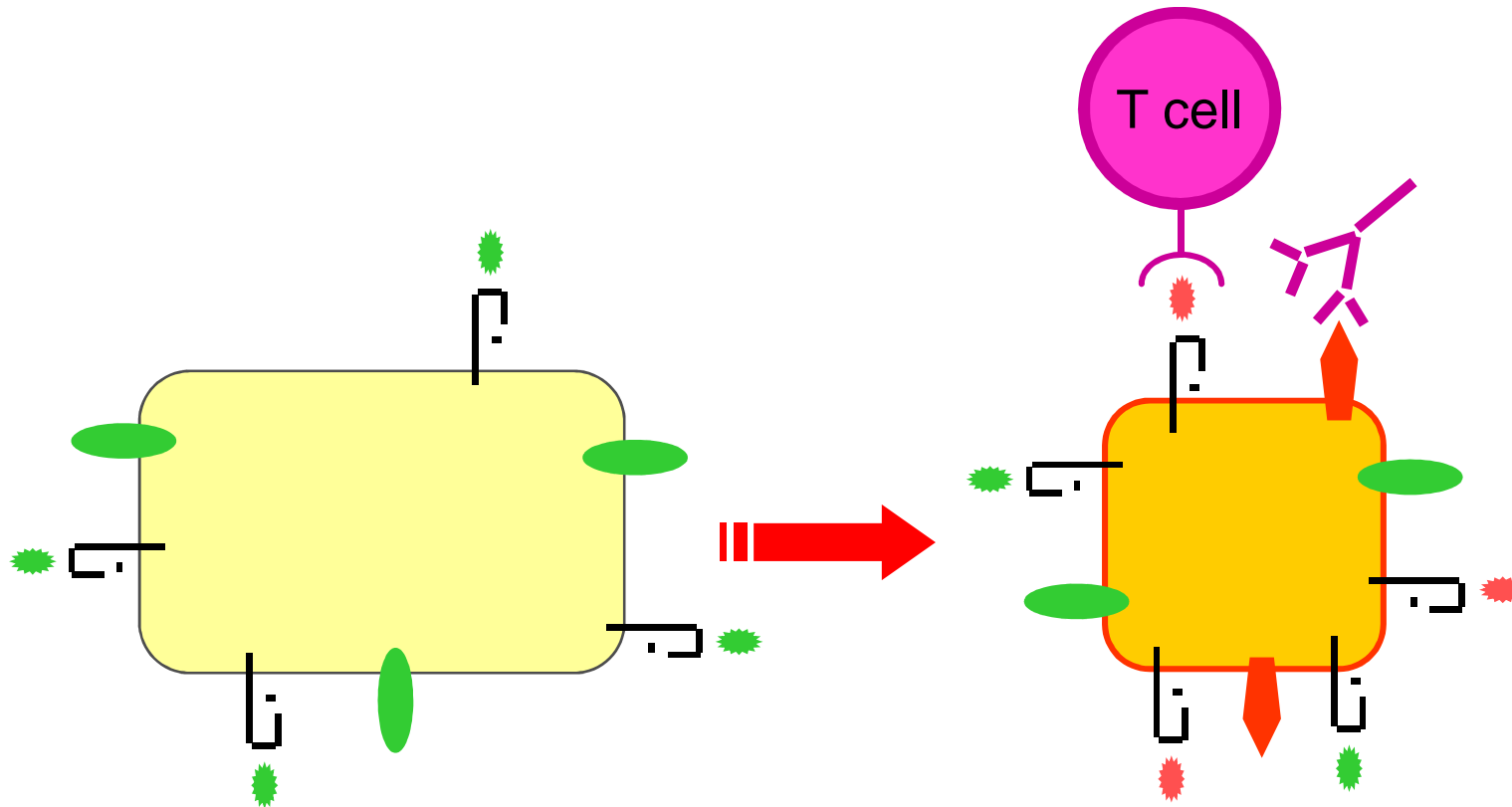
Restoration of HPV16-specific T-cell immunity through antigen-specific vaccination

	pre-vaccination				after 2 vaccinations				after 4 vaccinations					
	1	2	3	4	5	6	7	8	9	10	11	12		
A														
medium	0	2	1	0	2	0	0	4	1	0	3	2		
B														
HPV16 E6.1	1	0	1	2	14	18	8	8	29	20	24	23	} E6	
C														
HPV16 E6.2	2	2	1	3	27	23	21	16	60	67	43	52		
D														
HPV16 E6.3	1	2	2	3	46	35	54	55	106	94	82	97		
E														
HPV16 E6.4	1	0	0	0	1	4	0	1	0	3	1	1	} E7	
F														
HPV16 E7.1	2	1	6	5	3	1	1	3	1	0	2	1		
G														
HPV16 E7.2	0	0	1	1	19	13	14	24	114	110	118	89		

Rienk Offringa, Leiden, The Netherlands

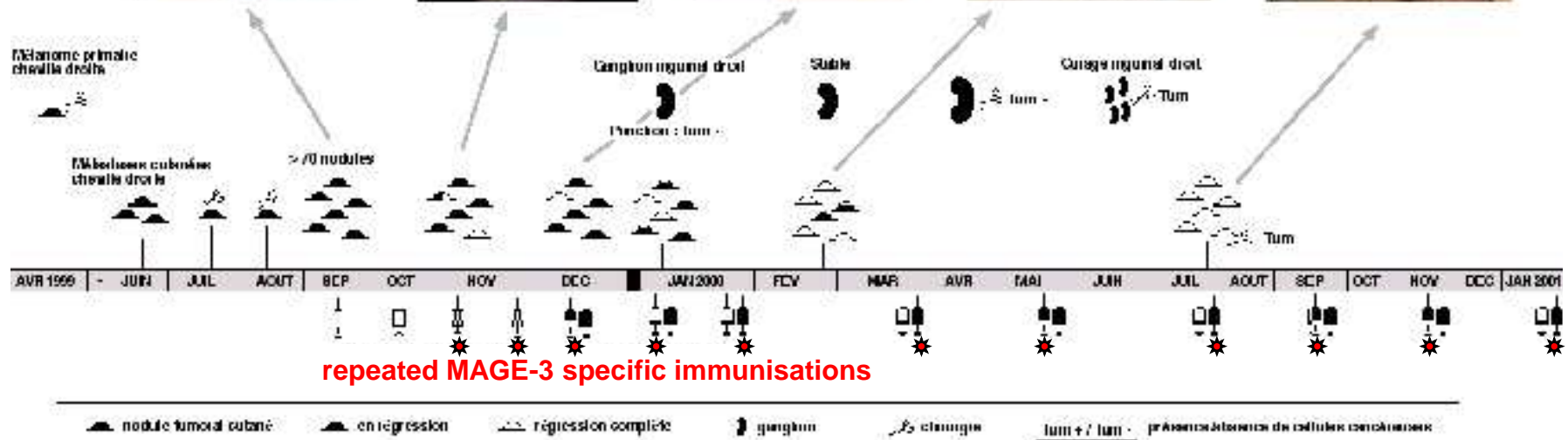
2

Tumour-associated antigens: ectopically expressed auto-antigens



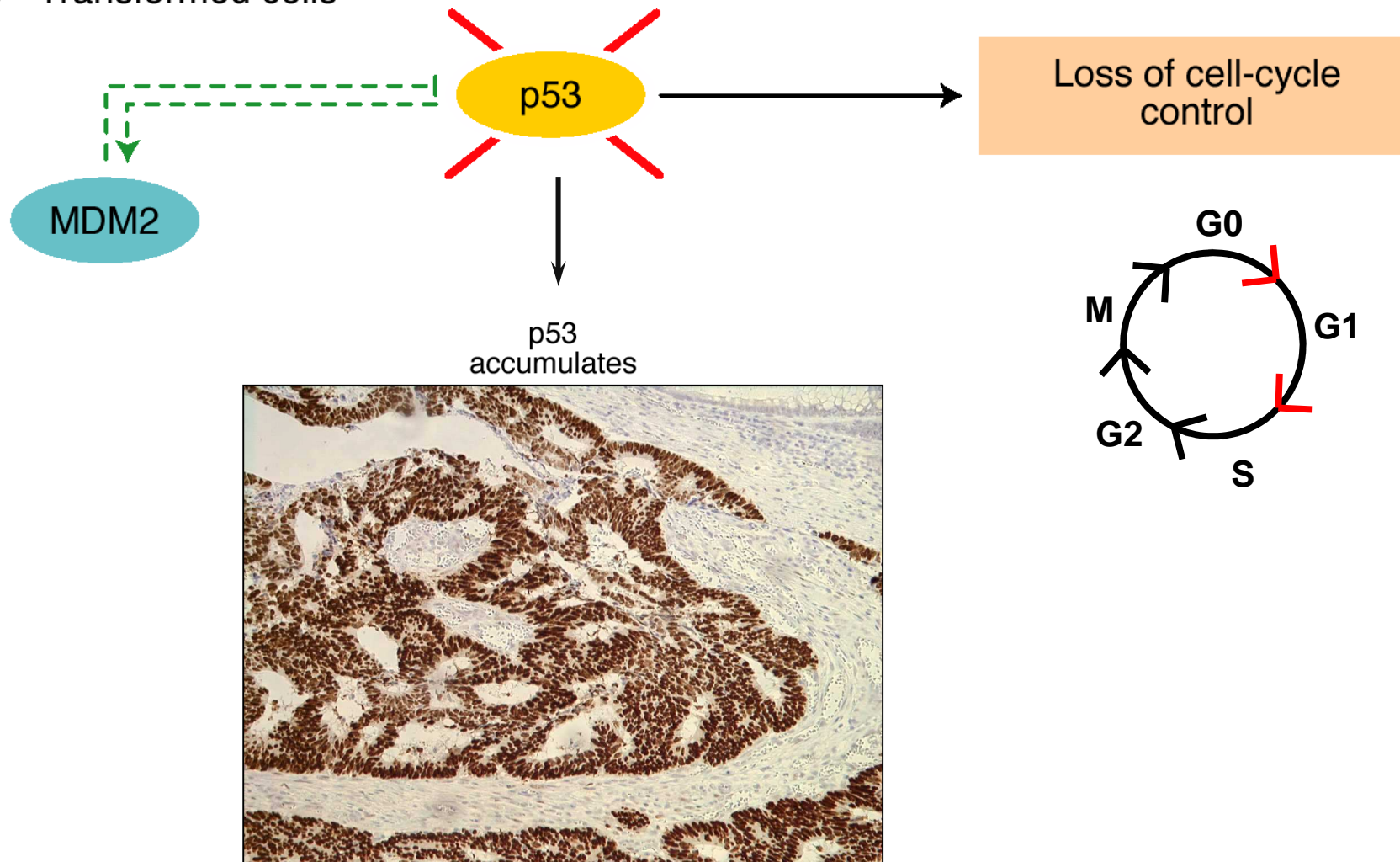
Cancer-testis antigens: e.g. MAGE-3

PATIENTE 0101



Other ectopically expressed auto-antigen: p53

c Transformed cells

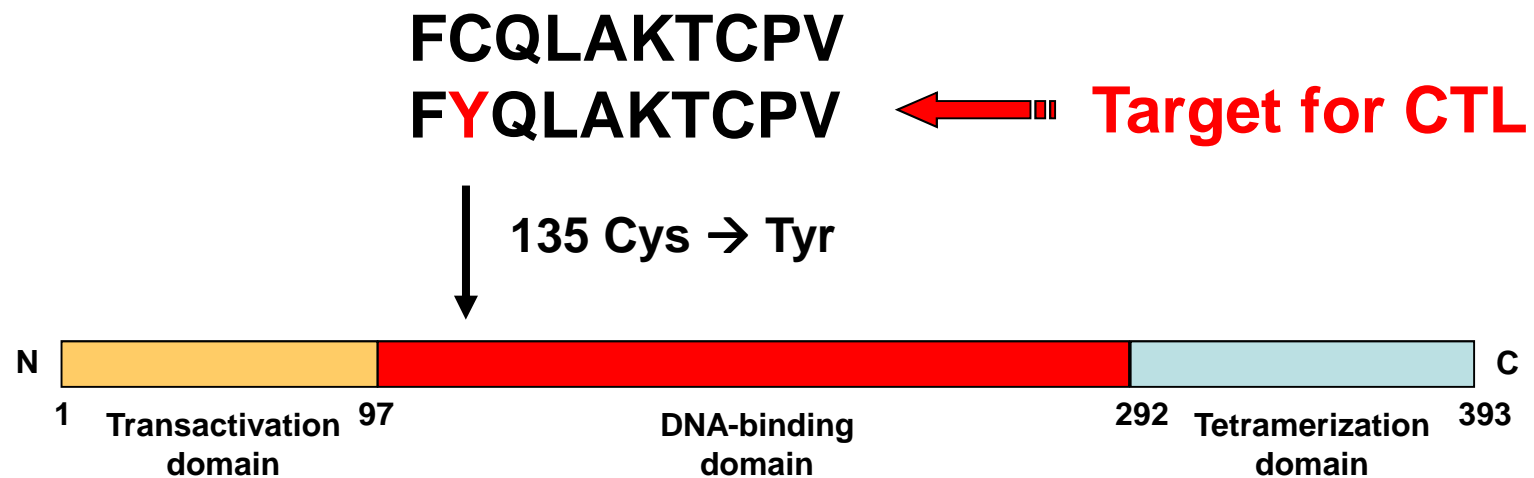


p53 is frequently mutated and overexpressed in human cancer

Cancer type	n	% mut. p53	Cancer type	n	% mut. p53	Cancer type	n	% mut. p53
Lung	897	56	Prostate	87	30	Carcinoid	61	11
Colon	960	50	HCC	716	29	Melanoma	70	9
Esophagus	270	45	Brain	456	25	Parathyr.	13	8
Ovary	386	44	Adrenal	31	23	Cervix	350	7
Pancreas	170	44	Breast	1536	22	Neurobl.	212	1
Skin	220	44	Endometr.	224	22	Wilms'	41	0
Gastric	314	41	Mesothel.	23	22	Testis	40	0
Head/Neck	524	37	Renal	102	19	Pitutary	27	0
Bladder	308	34	Thyroid	299	13	Pheochrom.	47	0
Sarcoma	339	31	Hemathol.	1916	12			

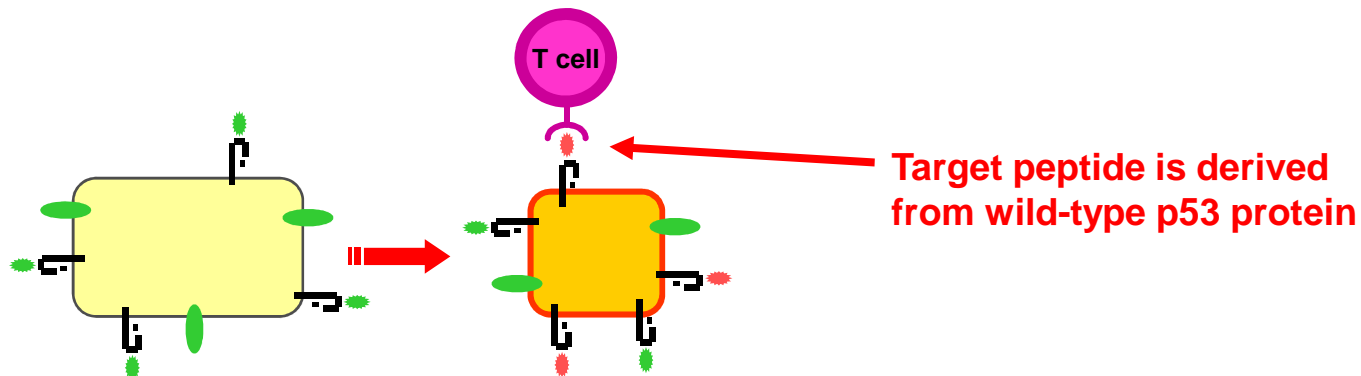
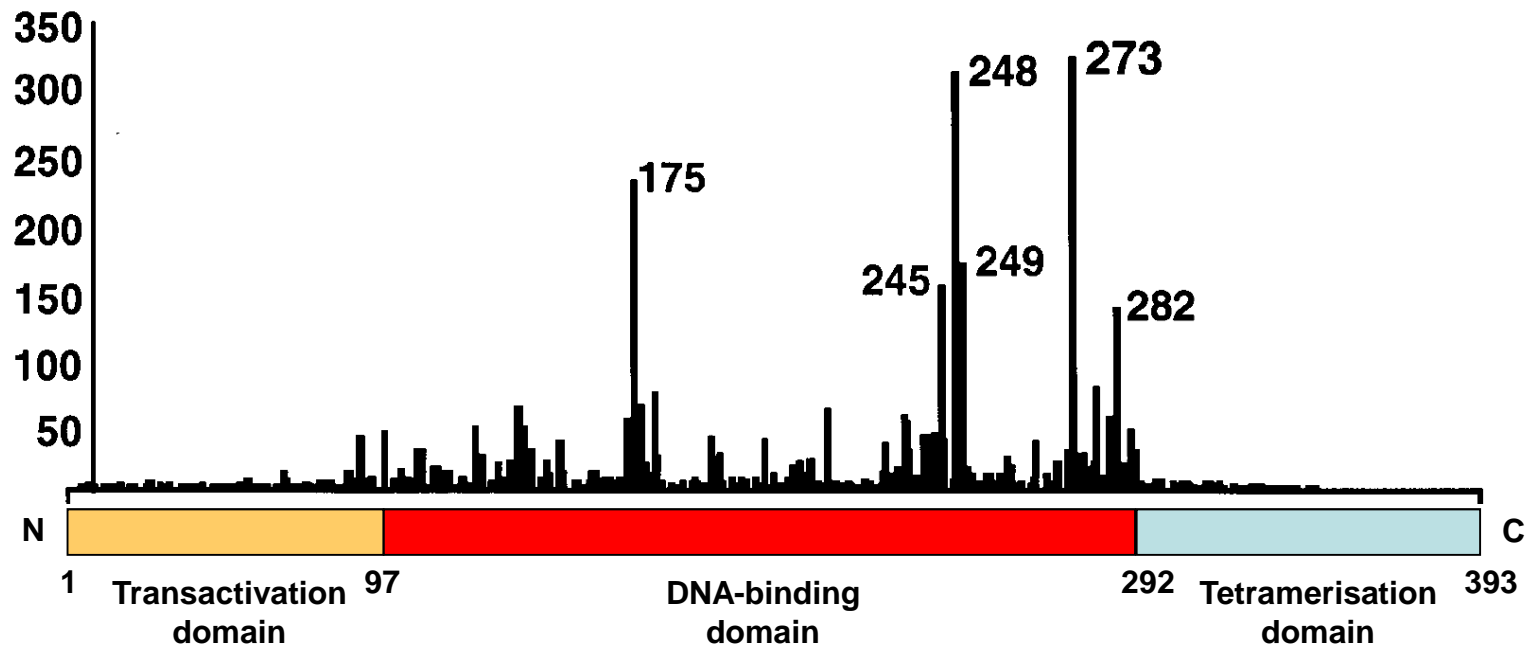
Source: Scientific American, October 1994)

Mutated p53 as a tumour-associated or tumour-specific antigen?

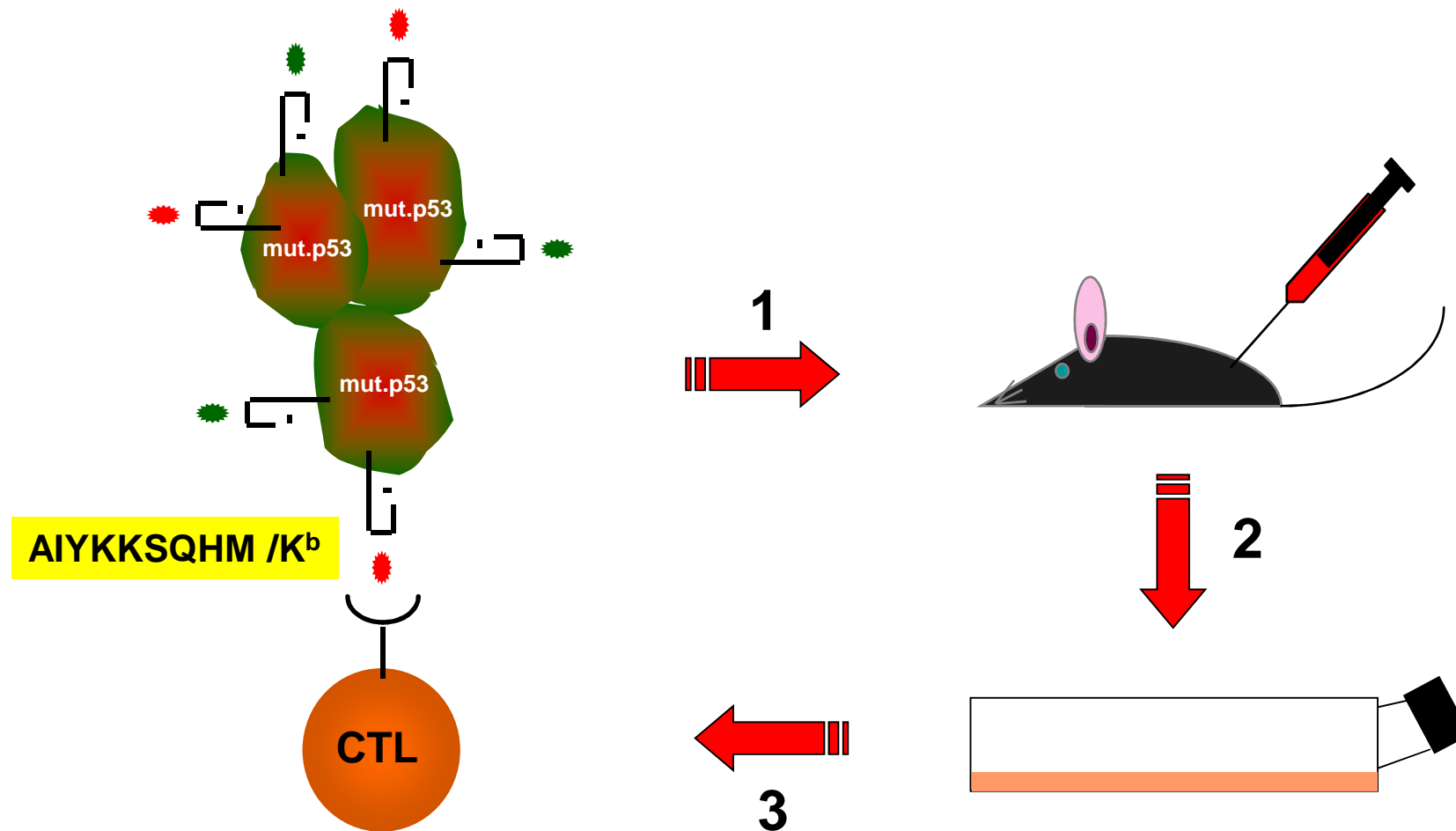


Yanuck et al. (1993) Cancer Res. 53, 3257

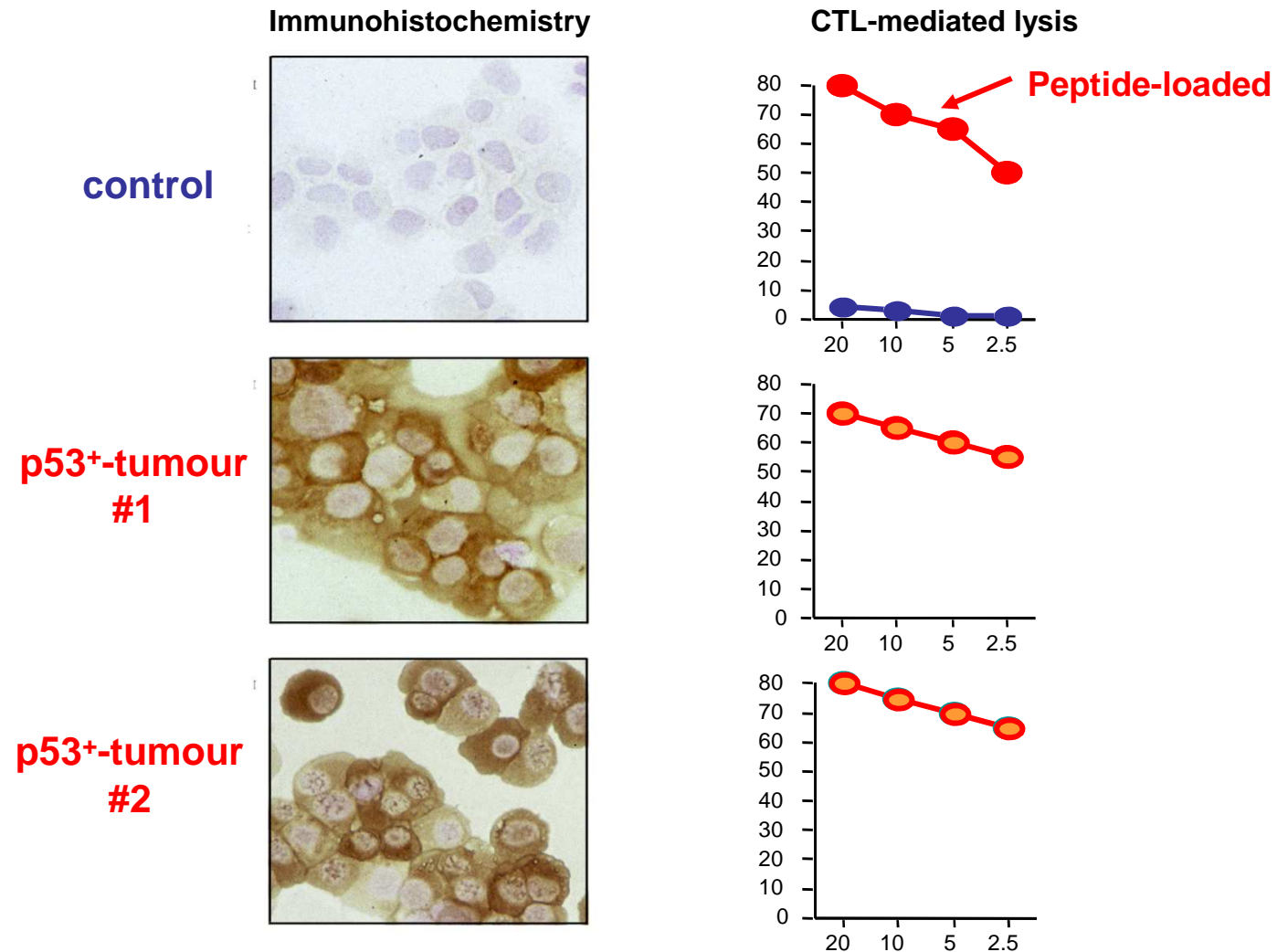
Mutations of p53 in human cancer are highly variable



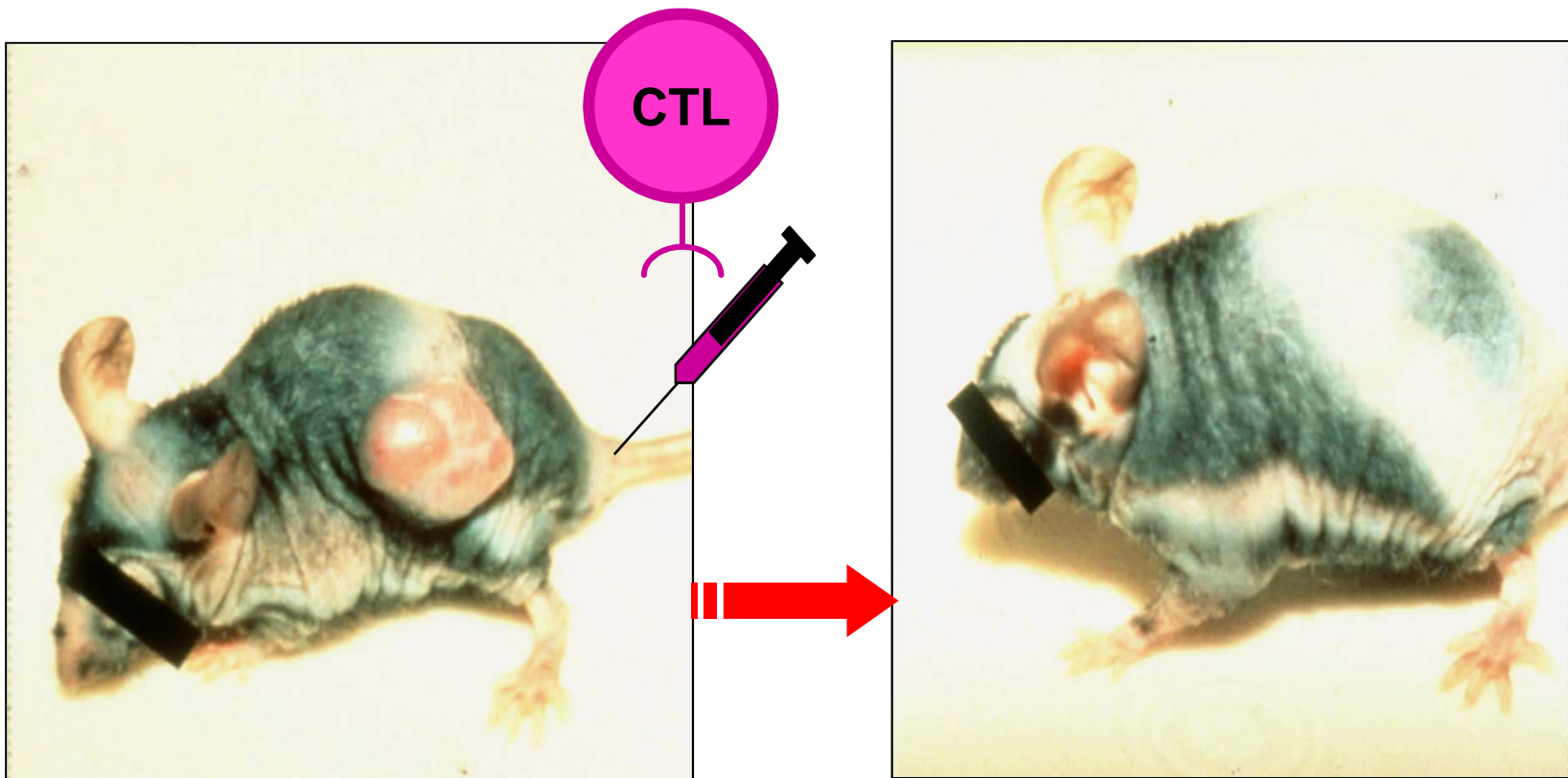
Isolation of p53-specific CTLs from spleens of immunised mice targeting **wild-type peptides of p53**



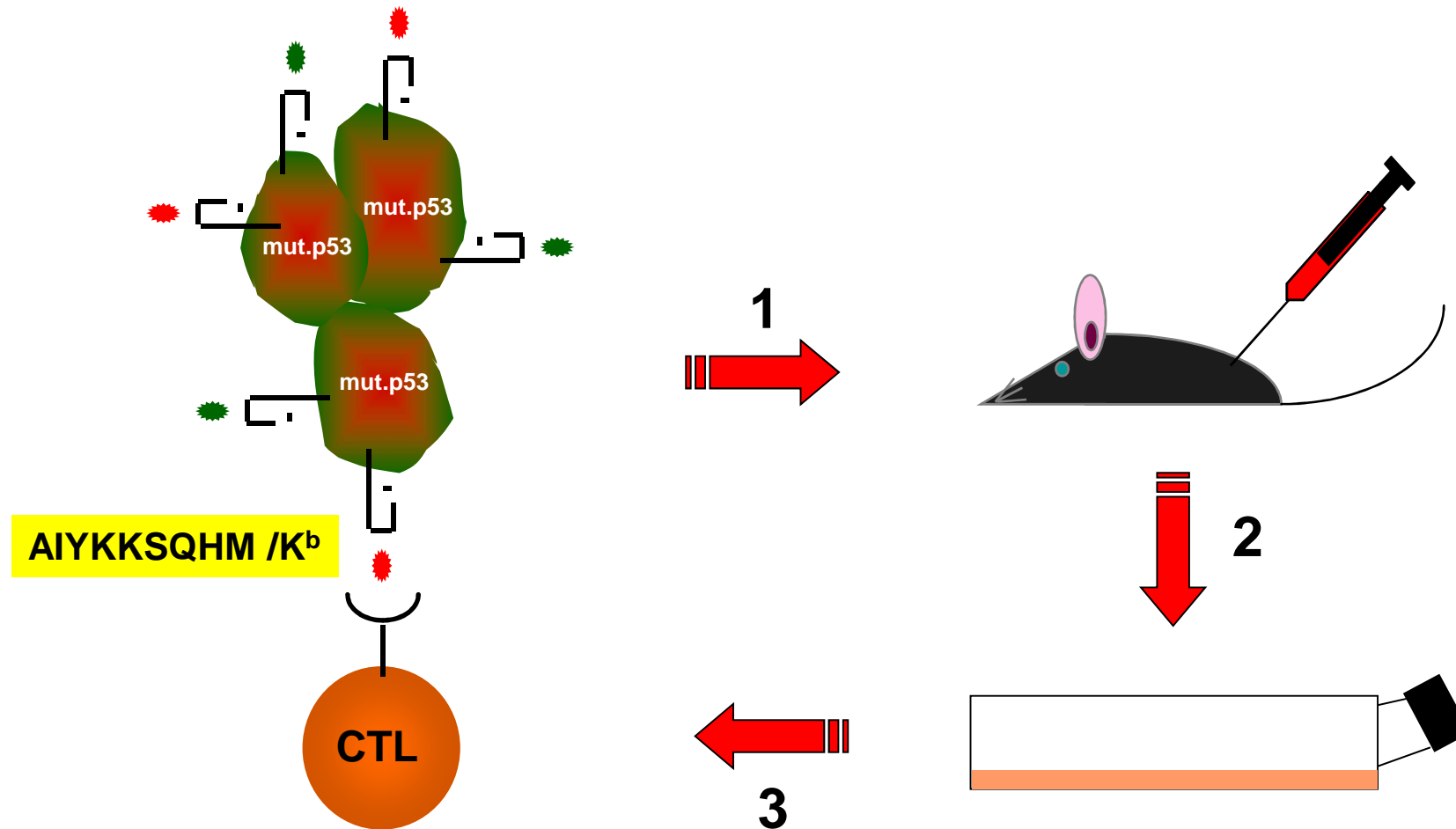
Proof-of-concept: CTLs directed against a **wild-type p53 peptide** are capable of selectively eliminating tumour cells *in vitro*



Successful cancer immunotherapy by adoptive transfer of *in-vitro*-expanded CTL specific for wild-type p53



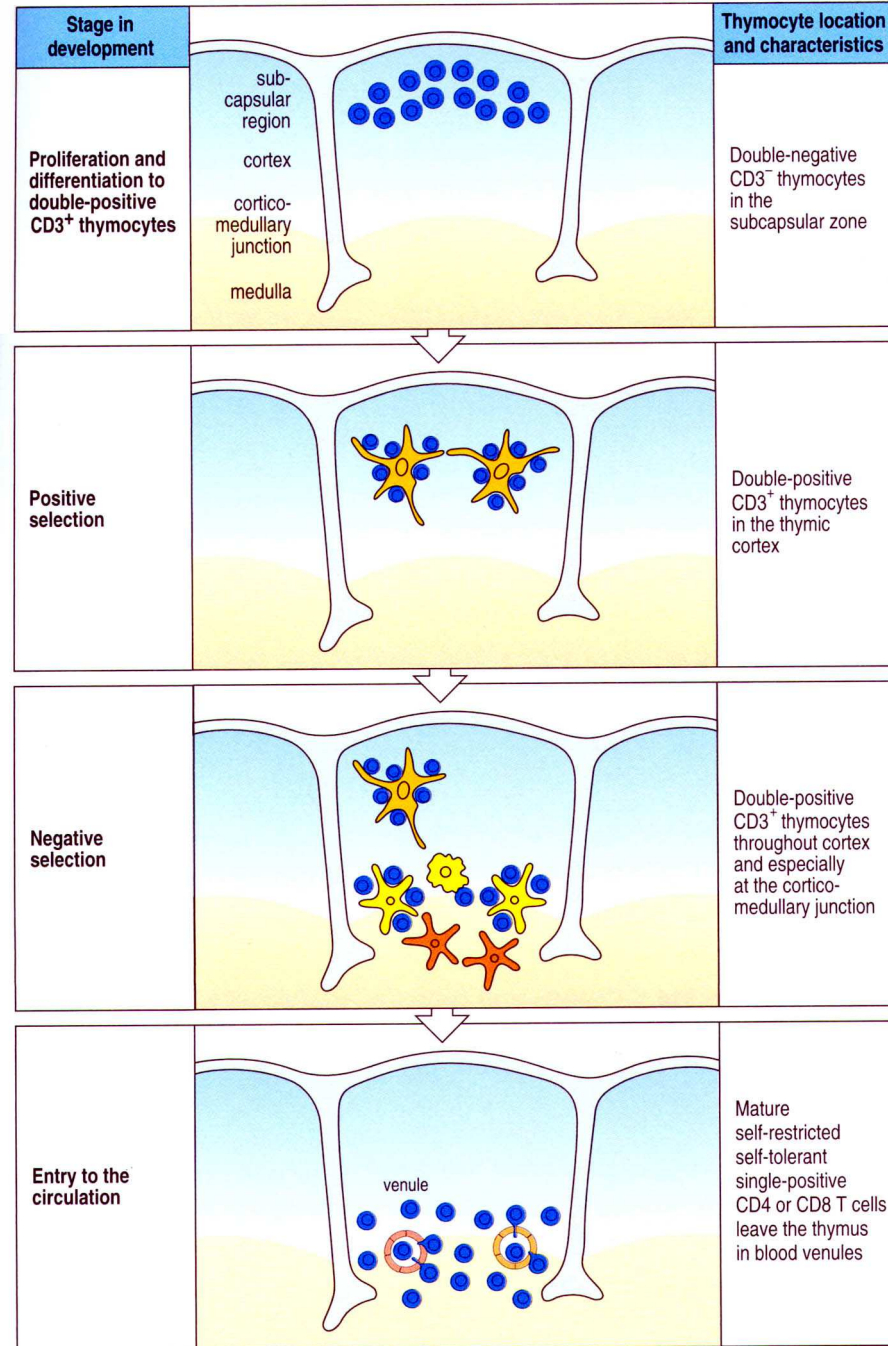
but... p53-specific CTL were isolated from
p53-deficient mice



We do not succeed in inducing such CTL in normal p53^{+/+} mice

Question: Why not?

Tolerance induction by negative selection in the thymus

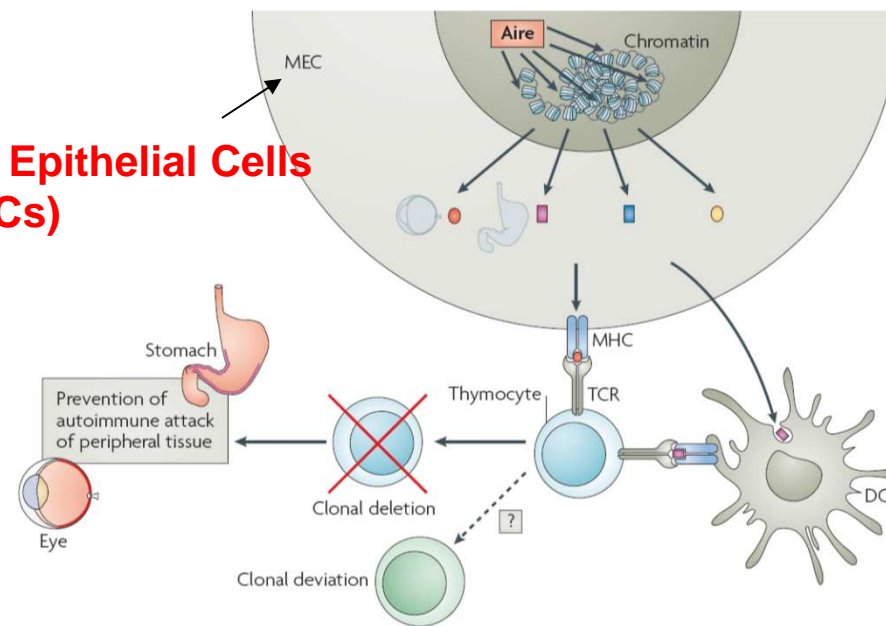


Tolerance induction by thymic negative selection

Central tolerance:

- **Ubiquitously** expressed self antigens (i.e. also in thymus)
- **AIRE-dependent** expression of tissue-specific self antigens* in thymus

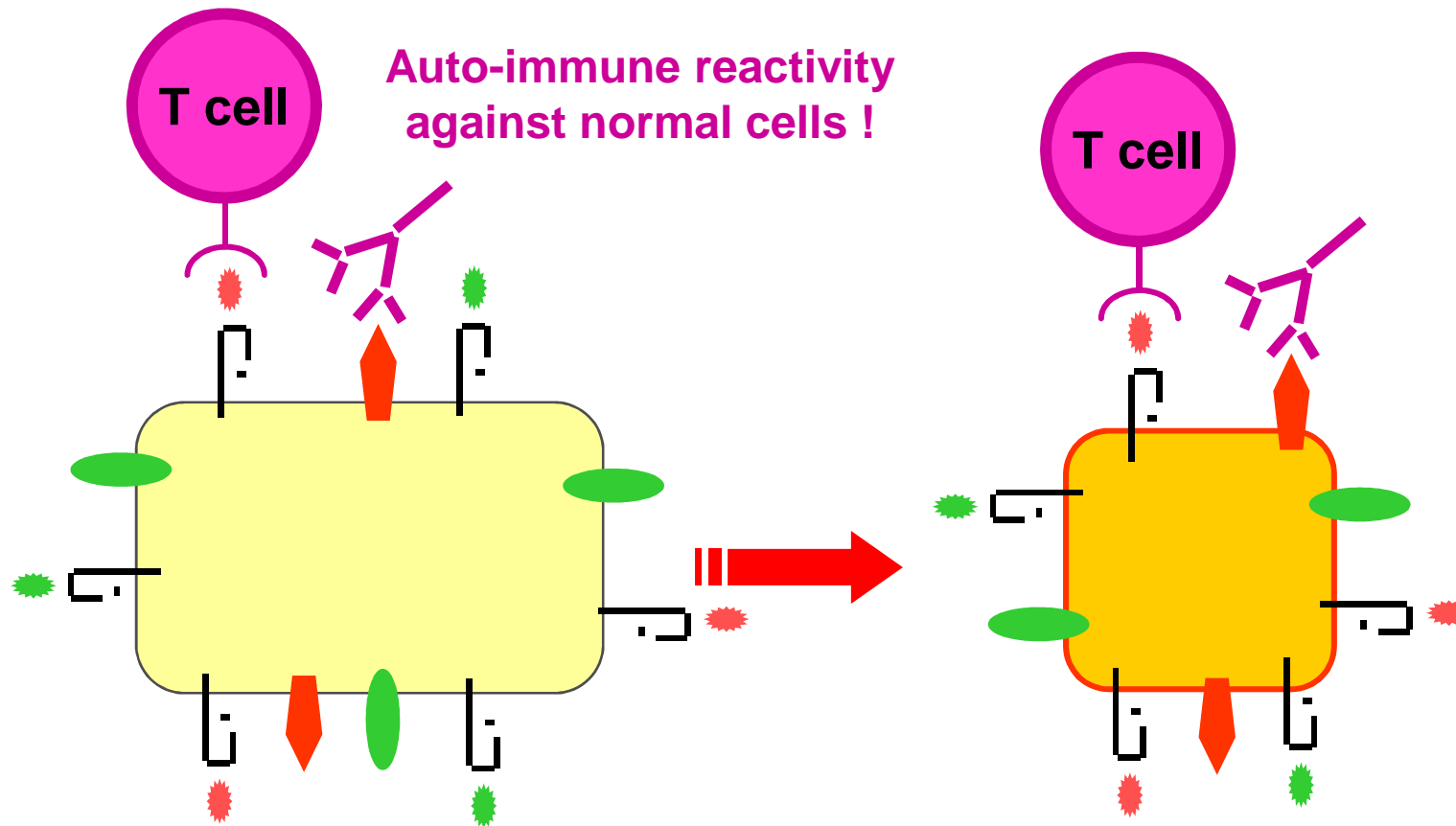
medullary Thymic Epithelial Cells (mTECs)



- * Expressed by **mTECs**:
- MART-1, tyrosinase
 - MAGE antigens
 - CEA

3

Tumour-associated antigens: differentiation (i.e. lineage-specific) auto-antigens



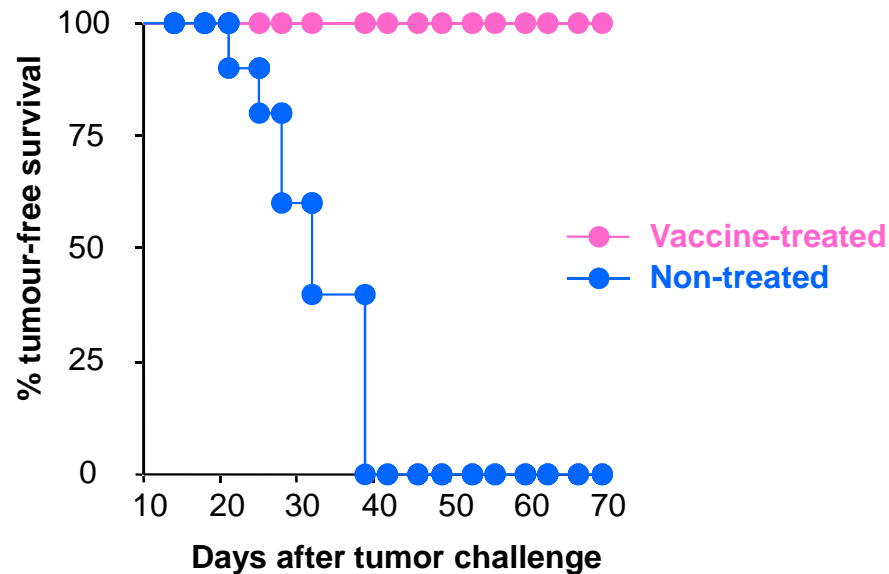
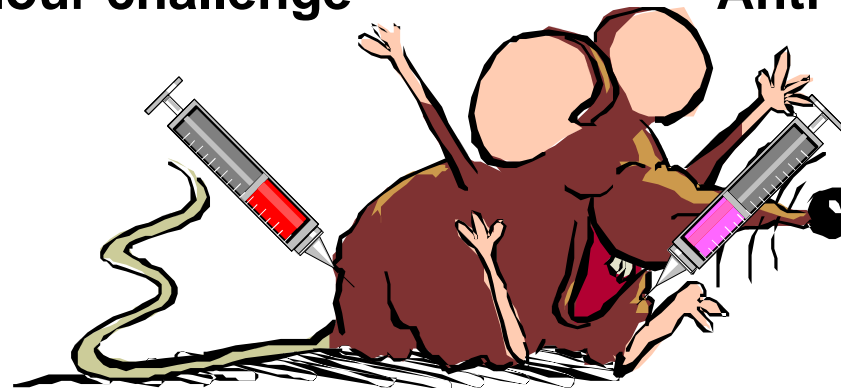
Melanocyte / melanoma – differentiation antigens
(e.g. tyrosinase)

Induction of auto-immune CTLs for therapy of experimental melanoma

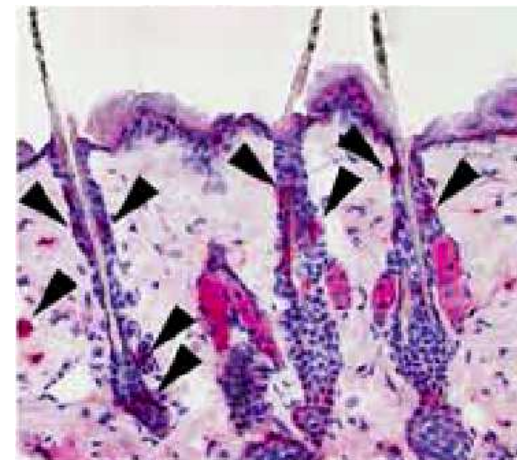
Van Elsas et al (1999) J Exp Med 190, 355

B16 tumour challenge

**Anti-tumour vaccination
(repeated)**

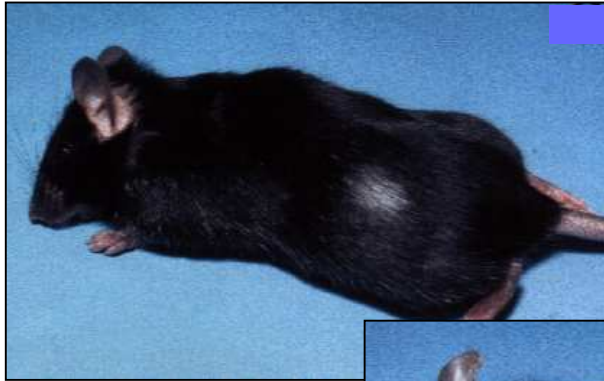


CTLs also attack normal melanocytes



Overwijk et al., unpublished

Immunotherapy against melanoma in mice is accompanied by auto-immune skin depigmentation

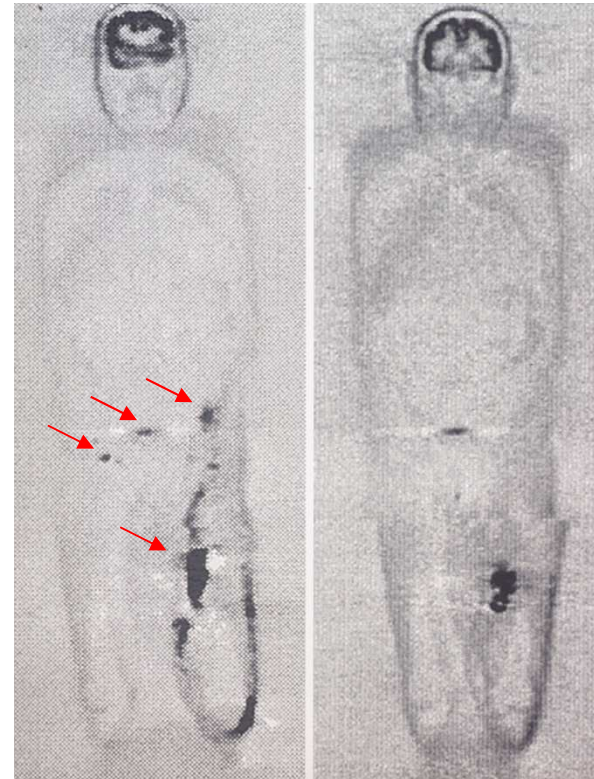


Van Elsas et al (1999)
J Exp Med 190, 355

Immunotherapy of melanoma in cancer patients: dendritic cells pulsed with tumour cell lysate (1)



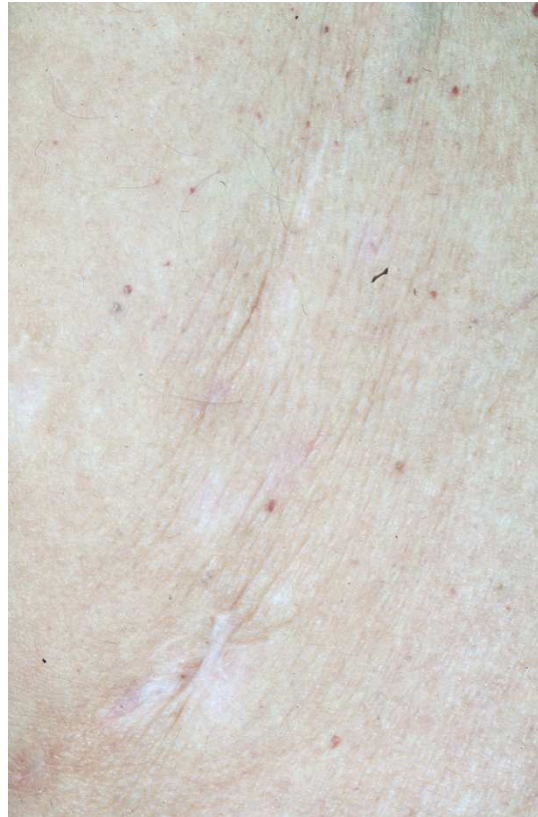
PET scan



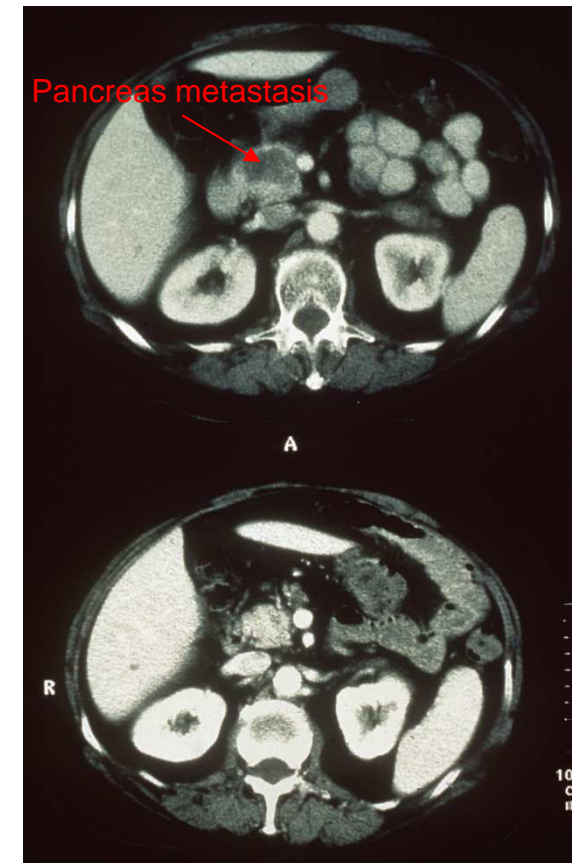
PR → CR

Courtesy of Frank Nestle, Zürich

Immunotherapy of melanoma in cancer patients: dendritic cells pulsed with tumour cell lysate (2)



CT scan, cross-section abdomen



PR

Courtesy of Frank Nestle, Zürich

Auto-immune depigmentation in melanoma patients



**Frank Nestle, Zürich
Switzerland**



**Nick Restifo & Steve Rosenberg
NIH, USA**

Targeting of tumour-associated auto-antigens for T cell-mediated immuno-therapy of cancer

Two major obstacles:

- 1. Auto-immune responses against normal tissues**

- 2. Immunological tolerance**
 - Normal tolerance to auto-antigens
 - Tumour-induced tolerance

Question:

Thymic tolerance prevents induction of CTL against the auto-antigen p53 in normal mice.

However, such CTL can be induced against other auto-antigens, e.g. Tyrosinase.

What could be the reason for this?

Another question:

Immunotherapy against cancer can be associated with auto-immunity against normal somatic tissues.

In the case of melanoma this is a nuisance, but not dangerous.

In which cases would this be dangerous?

Conclusions

- **Preventive and therapeutic immune intervention against virus-induced cancers comes within reach**
- **Immunotherapy of non-virally induced cancers:**
 - ❖ **First promising results in clinical studies have been obtained... however, complete, and hence irreversible, tumour rejection is still rare.**
 - ❖ **Immunological tolerance, induced by normal tissues or by the tumour itself, still constitutes a major obstacle.**

What you should now be able to do

- ❖ **Outline evidence for the importance of tumour immune surveillance by the immune system**
- ❖ **understand that immune responses to tumours have some similarities with those to virus-infected cells**
- ❖ **Explain the concept of tumour-associated antigens**
- ❖ **Outline approaches that are being developed for tumour immunotherapy**

Credits

**University Hospital of Zürich
Switzerland:**

Frank Nestle

**Institute Pasteur
Paris, France:**

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