*Medical Biotechnology 2018'*

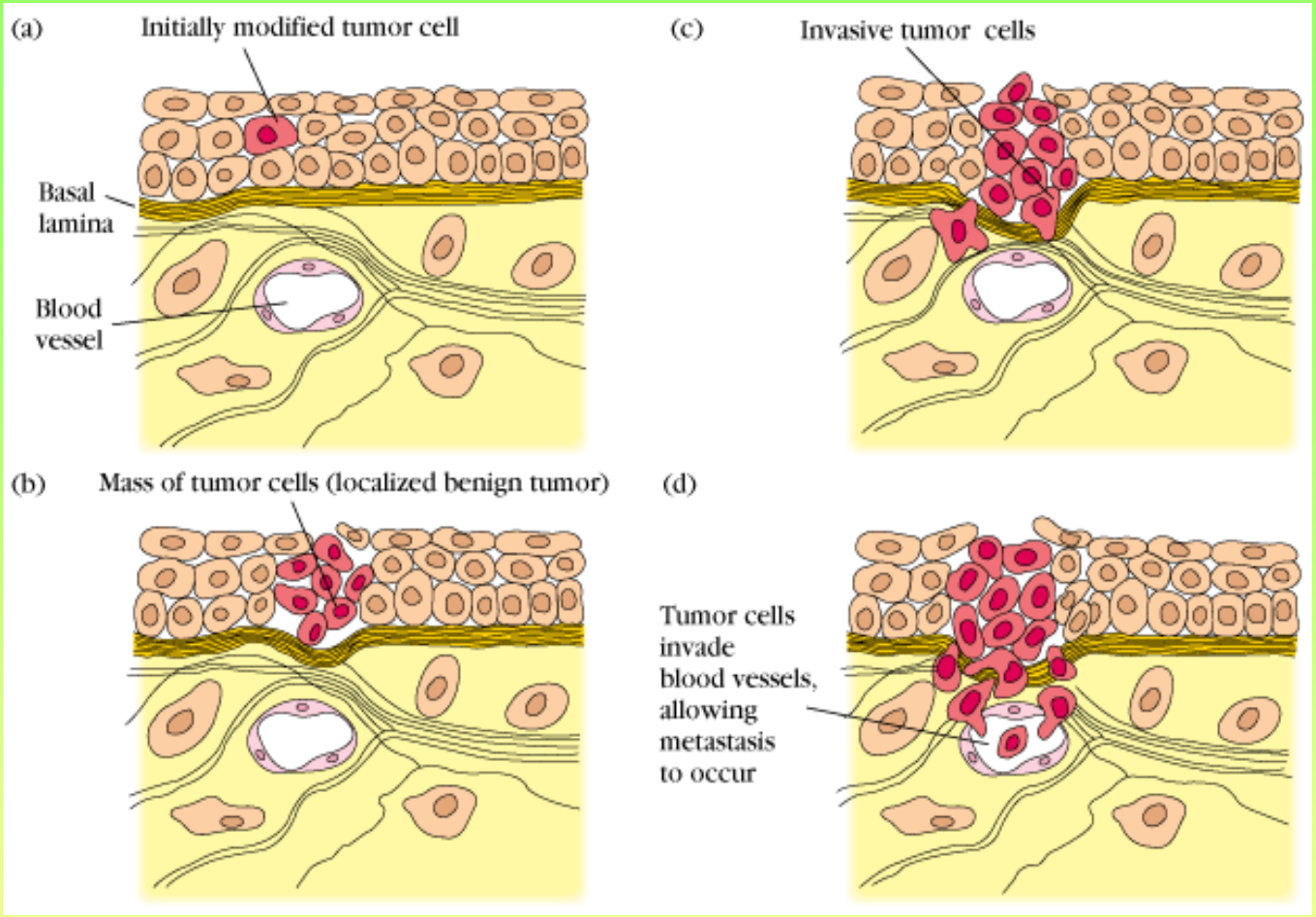
# Biological therapies

## Lecture 13-14th

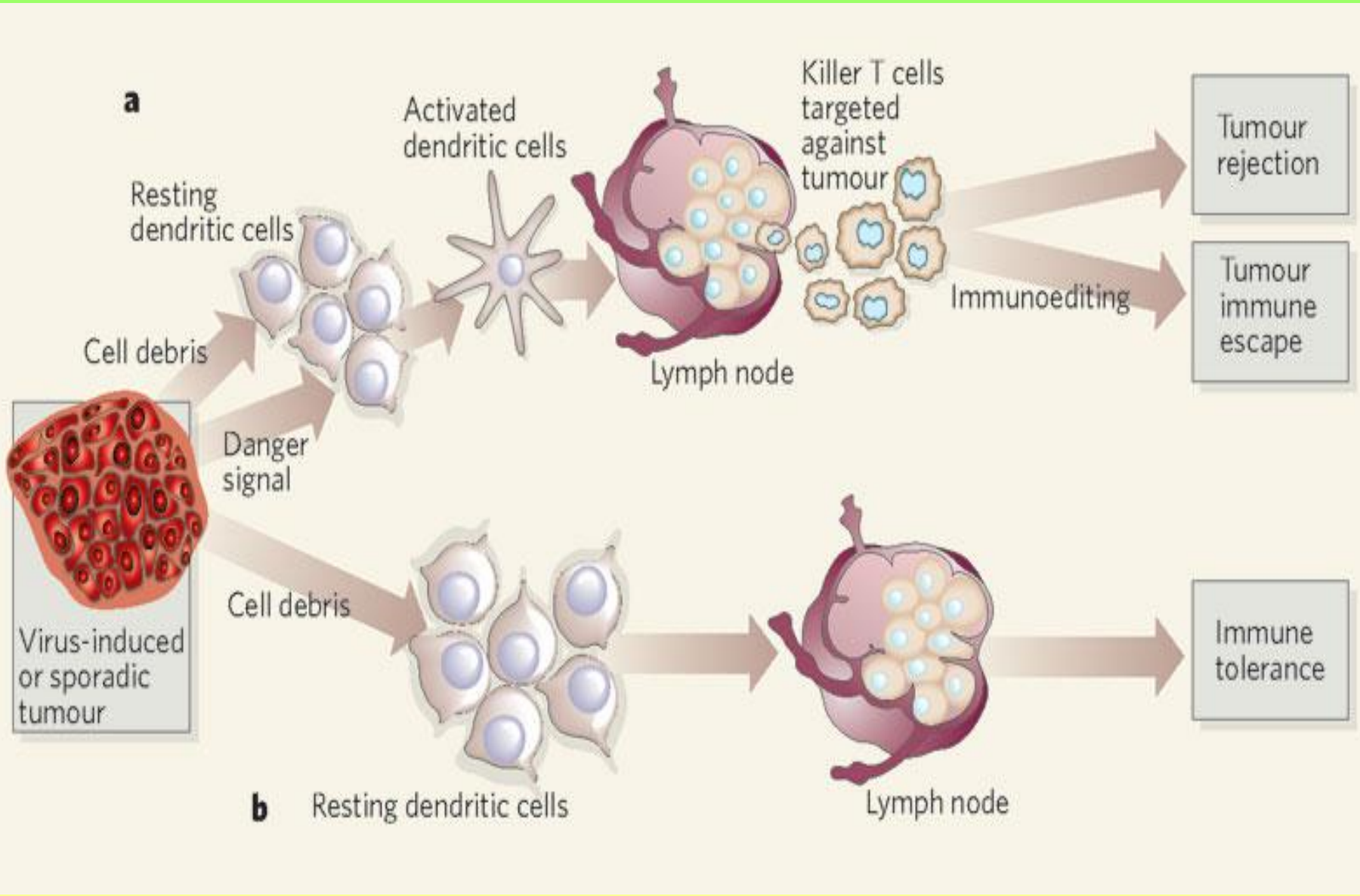
## Introduction into the biotherapies of malignant tumors

# History

- Tumor immunotherapy: ***William Coley*** an American bone surgeon (1862-1936) used Streptococcus pyogenes injection into bone tumours (TNFalpha induction)
- Idea of combination of bio- and chemotherapy: ***Paul Ehrlich*** (1854-1915): “magic bulet” (therapeutic monoclonal antibodies)
- Tumor vaccines and gene therapy: ***Steven Rosenberg*** (Nobel prize in 2012) pioneered the development of effective immunotherapies and gene therapies for patients with advanced cancers.

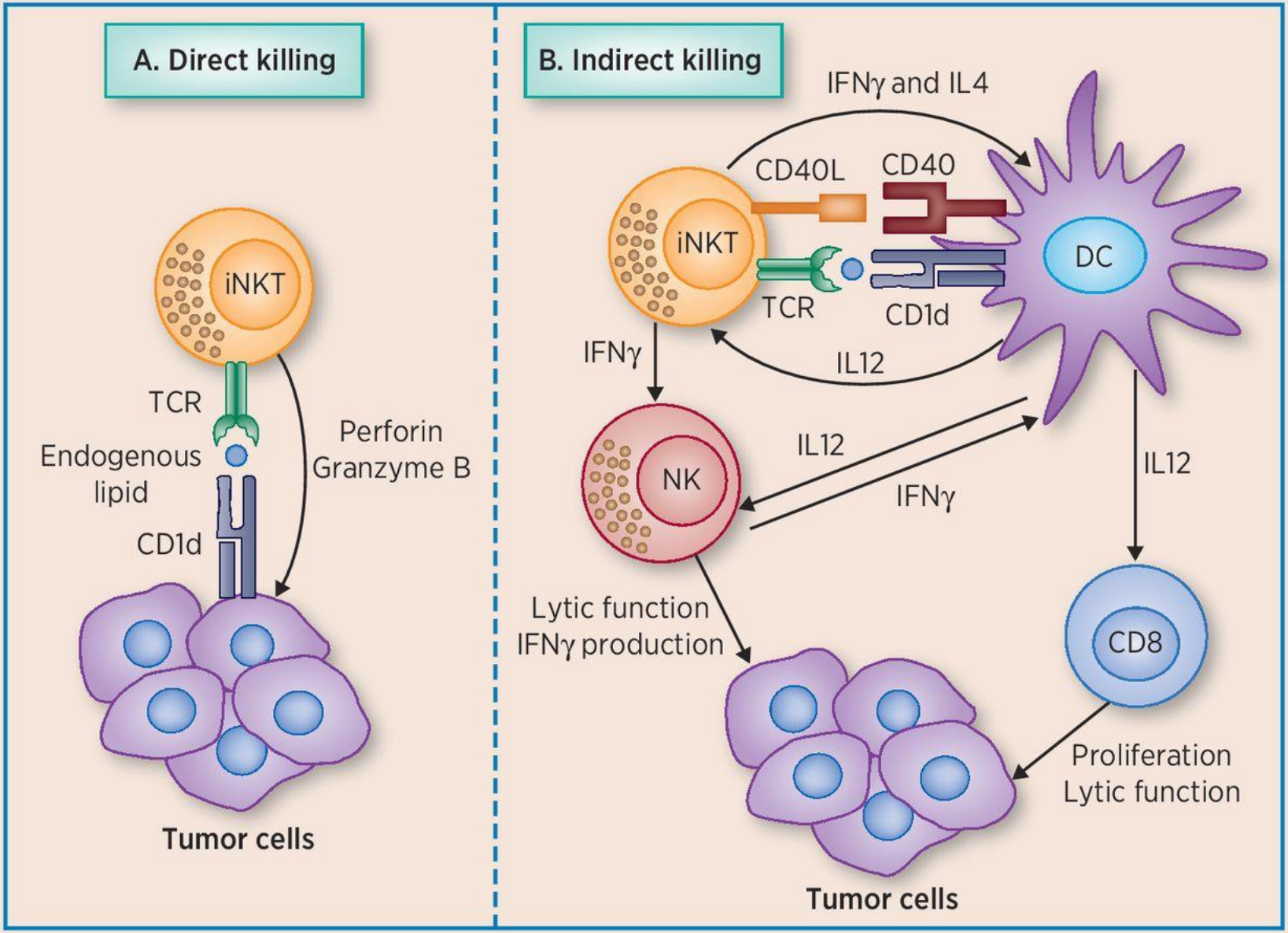



- Carcinogenesis is a multistep process with accumulation of multiple mutations
- Non-lethal genetic damages
- Clonal expansion (tumors are monoclonal)
- Tumor development (tumor escape or involvement)


a
Activated dendritic cells
Killer T cells targeted against tumour
Tumour rejection
Resting dendritic cells
Tumour immune escape
Immunoediting
Cell debris
Lymph node
Danger signal
Virus-induced or sporadic tumour
Cell debris
Immune tolerance
b
Resting dendritic cells
Lymph node

# Immune response against tumors

- Components of both innate, natural and adaptive immunity (iNKT, MAIT, iγδT cells)
- T cell mediated immune response (CD8+, CD4+Th1, γδT cells, NK, NK T cells)
- Macrophage mediated immune response
- immunoglobulin mediated (ADCC)
- Network of cytotoxic cytokines



# Tumor Specific Antigen

- **TSA – mutations of somatic cells induced by chemical carcinogenesis, viruses or x-rays**

- **Each carcinogenic factor induces a unique and specific class of antigens.**

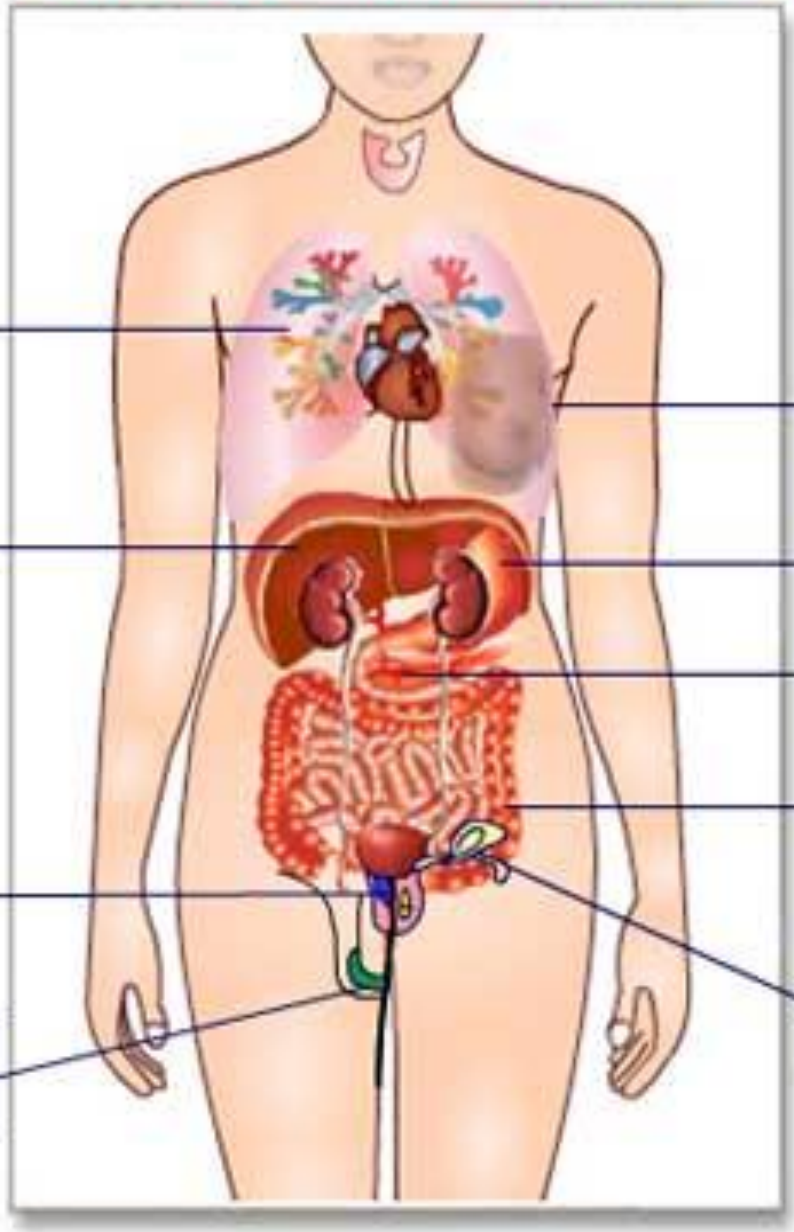
- **TSA is the result of somatic mutations which is recognized (according to the individual MHC haplotype) by the immune system.**

# Tumor Associated Antigen

**Products (e.g. hormones, growth factors, cell surface receptors, differentiation molecules etc.) of both normal and altered cells during their differentiation.**

**Production of TAAs is not related with tumorous transformation exclusively, but expression profile of TAAs could be characteristic in some tumours, and useful as „tumor markers" in differential diagnosis or in the monitoring of therapeutic efficiency.**

# Clinical Tumor Markers



Lung Cancer
CA125,CEA

Liver Cancer
AFP

Prostate Cancer
PSA

Testicular Cancer
AFP,HCG

Breast Cancer
CA125,CEA,HER2

Stomach Cancer
CEA

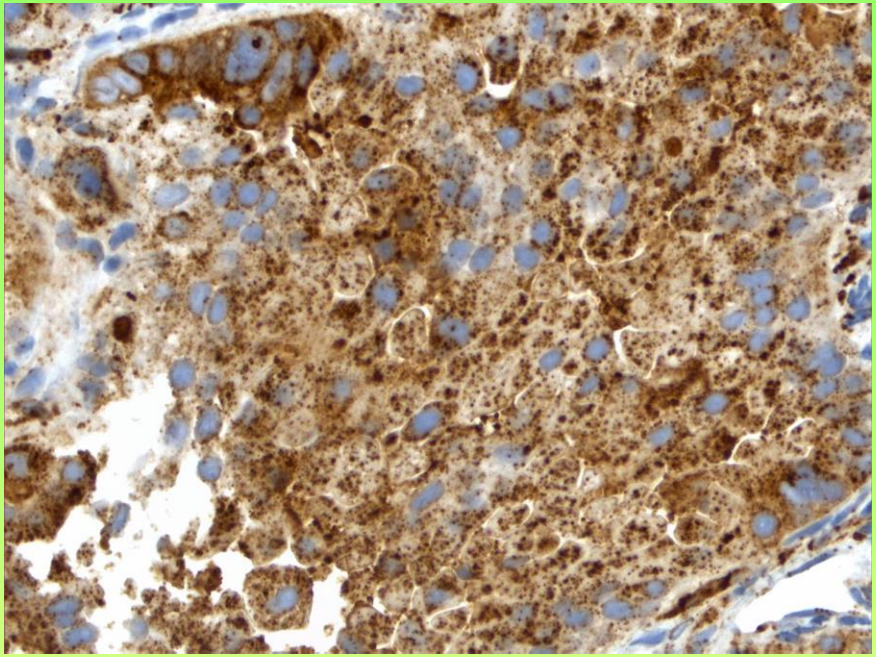
Pancrease Cancer
CA125,CEA

Colon Cancer
CEA

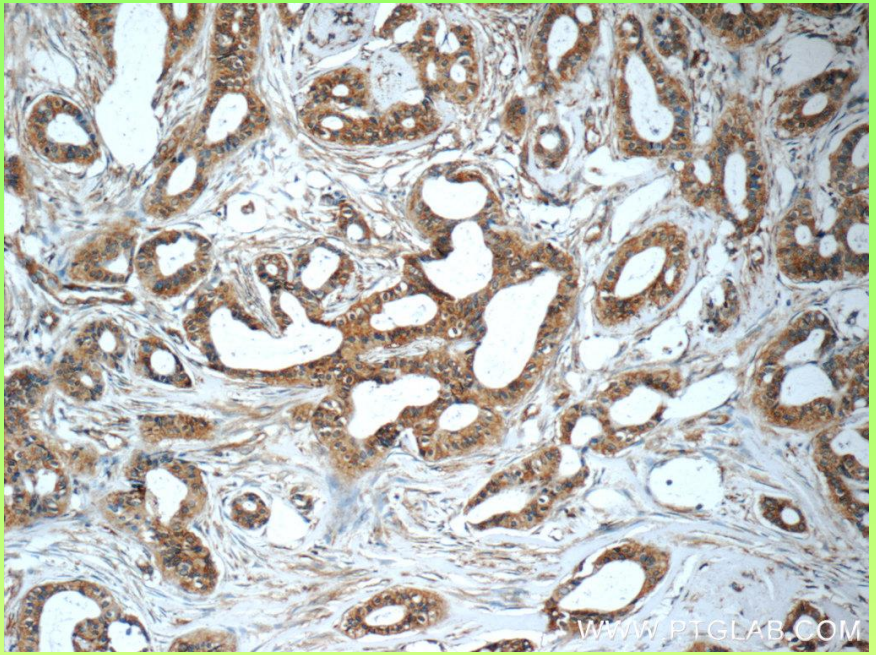
Ovaries Cancer
CA125,CEA

# Often tumor markers

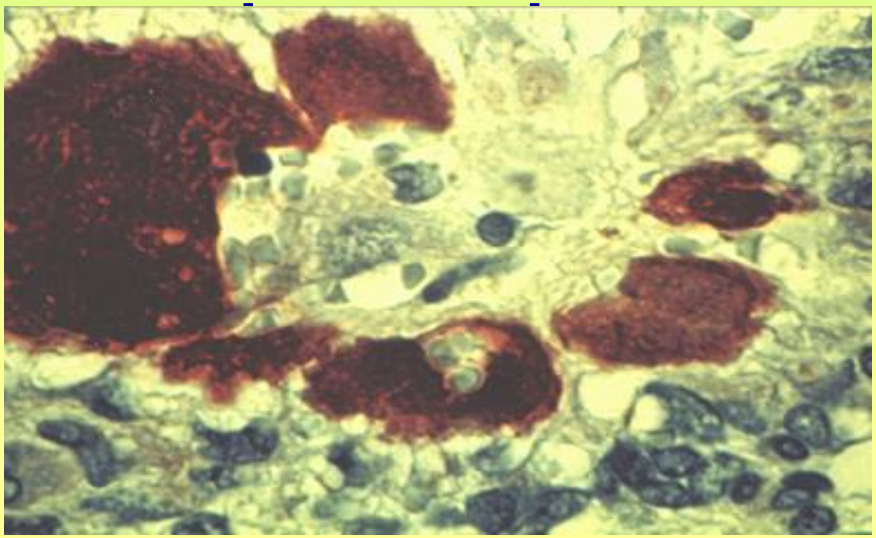
| Tumor markers | Abbreviation | Oncological application |
|---|---|---|
| Alfa-foetoprotein | AFP | Liver and germ cell tumors |
| Cancer antigen 125 | CA 125 | ovarian tumors |
| Cancer antigen 15,3 | CA 15,3 | Breast cancer |
| Cancer antigen 72,4 | CA 72,4 | Gastric cancer |
| Cancer antigen 19,9 | CA 19,9 | Pancreatic cancer |
| Carcinoembrional antigen | CEA | Gastrointestinal cancers |
| Neuronspecific enolase | NSE | Small cell lung cancer |
| Prostate specific antigen | PSA | Prostate cancer |
| Squamous cell carcinoma antigen | SCC | Planocellular cancers |
| Tissue polypeptide antigen | TPA | Urinary bladder and lung cancer |
| Tissue polypeptide-specific antigen | TPS | Metastatic breast cancer |



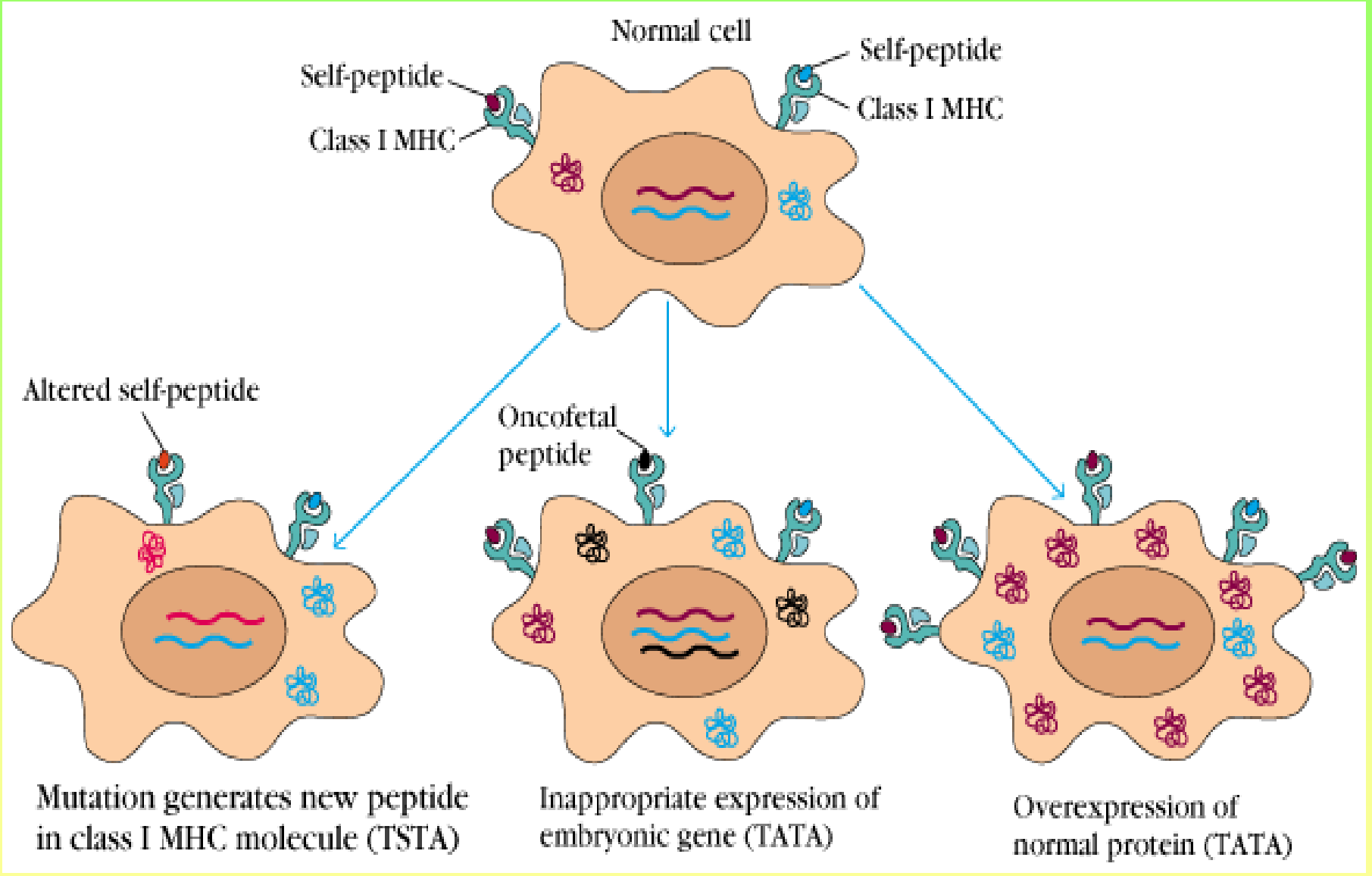
**Naspin A in bone metastasis of lung**




**LGALS3BP Galectin 3 binding in breast cancer metastasis**



**hCGβ in choriocarcinoma**


Normal cell
Self-peptide
Class I MHC
Self-peptide
Class I MHC
Altered self-peptide
Oncofetal peptide
Mutation generates new peptide in class I MHC molecule (TSTA)
Inappropriate expression of embryonic gene (TATA)
Overexpression of normal protein (TATA)

**Tumor-specific antigen –** antigen expressed on tumor cells but not on normal cells.

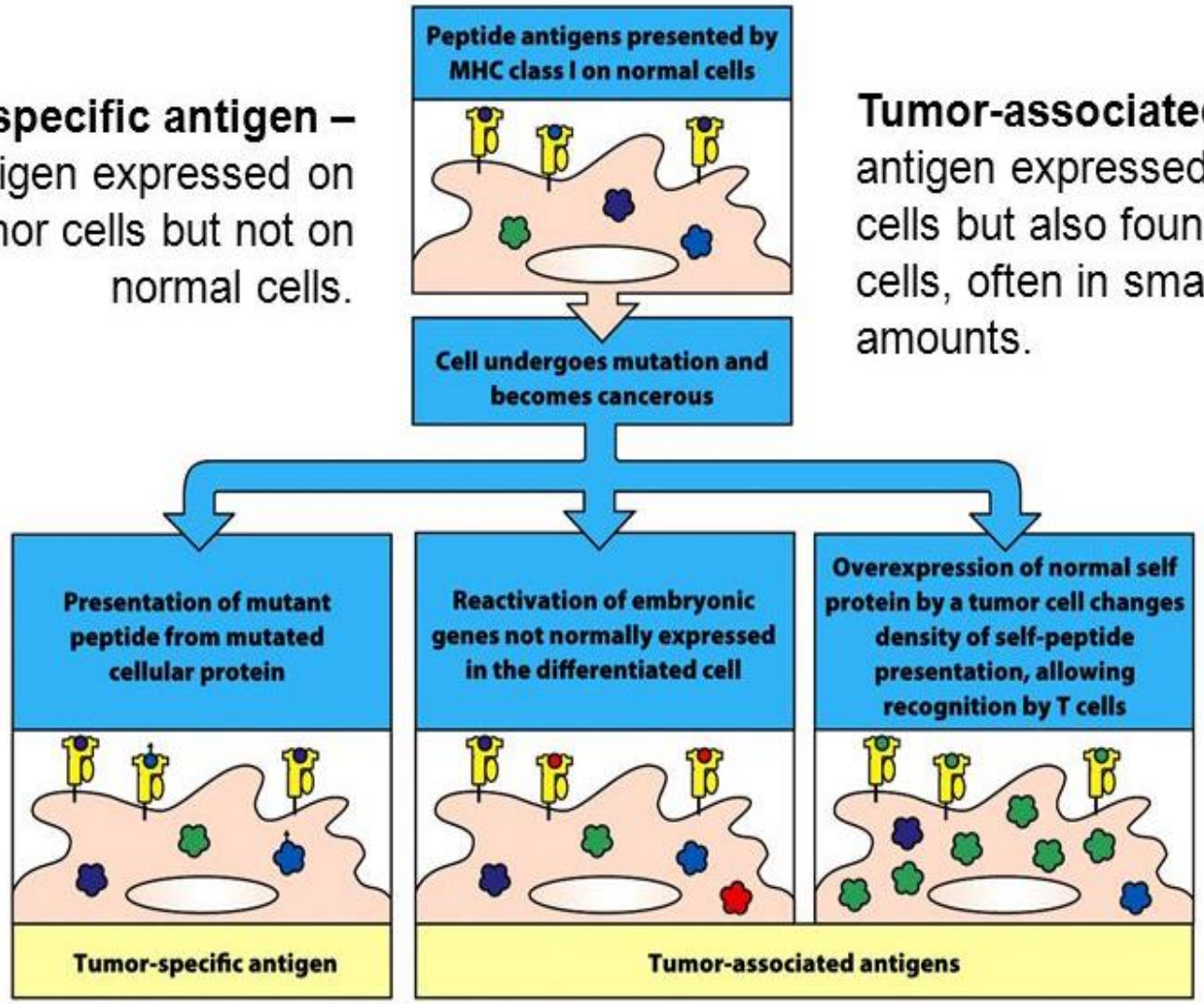
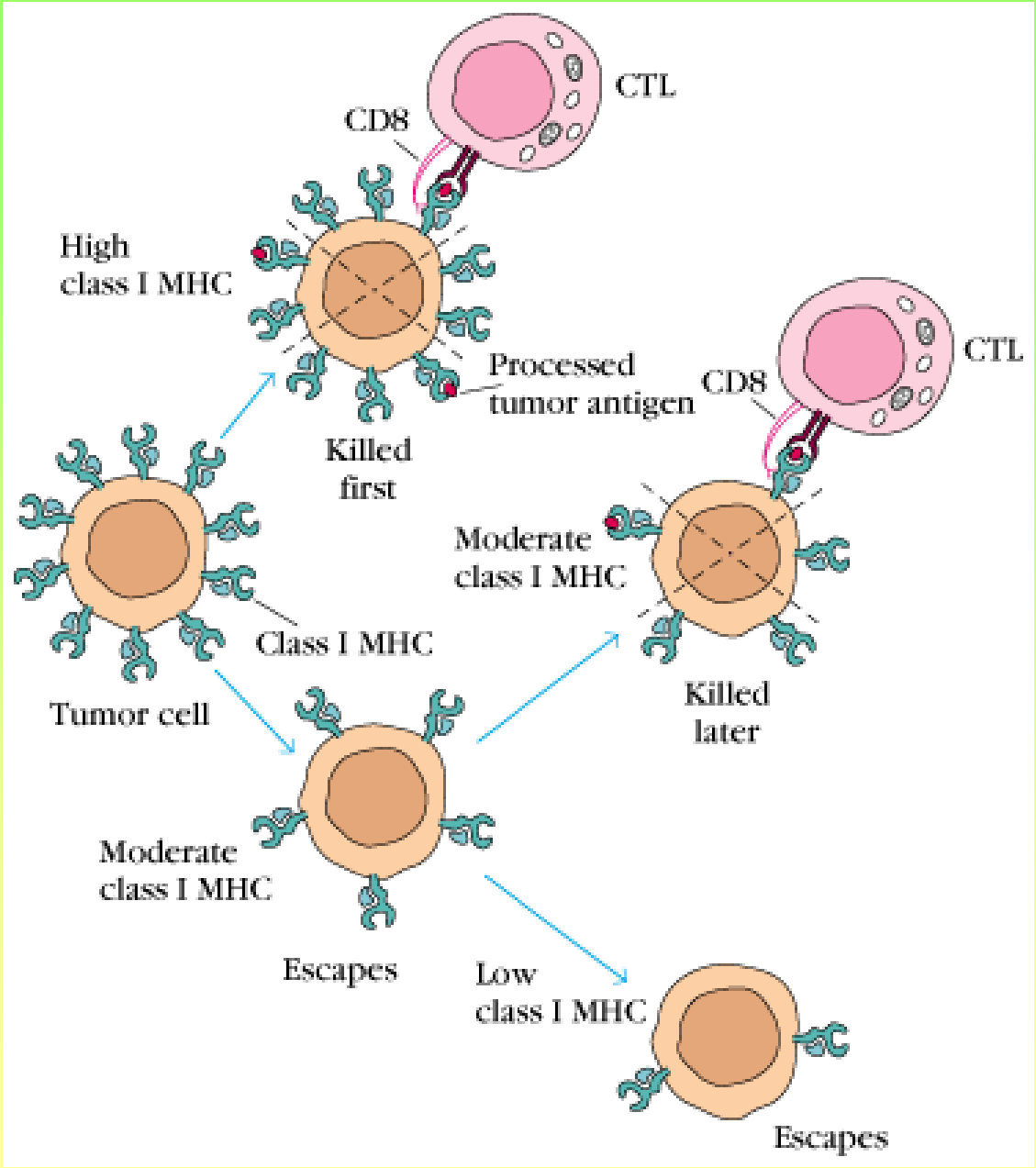


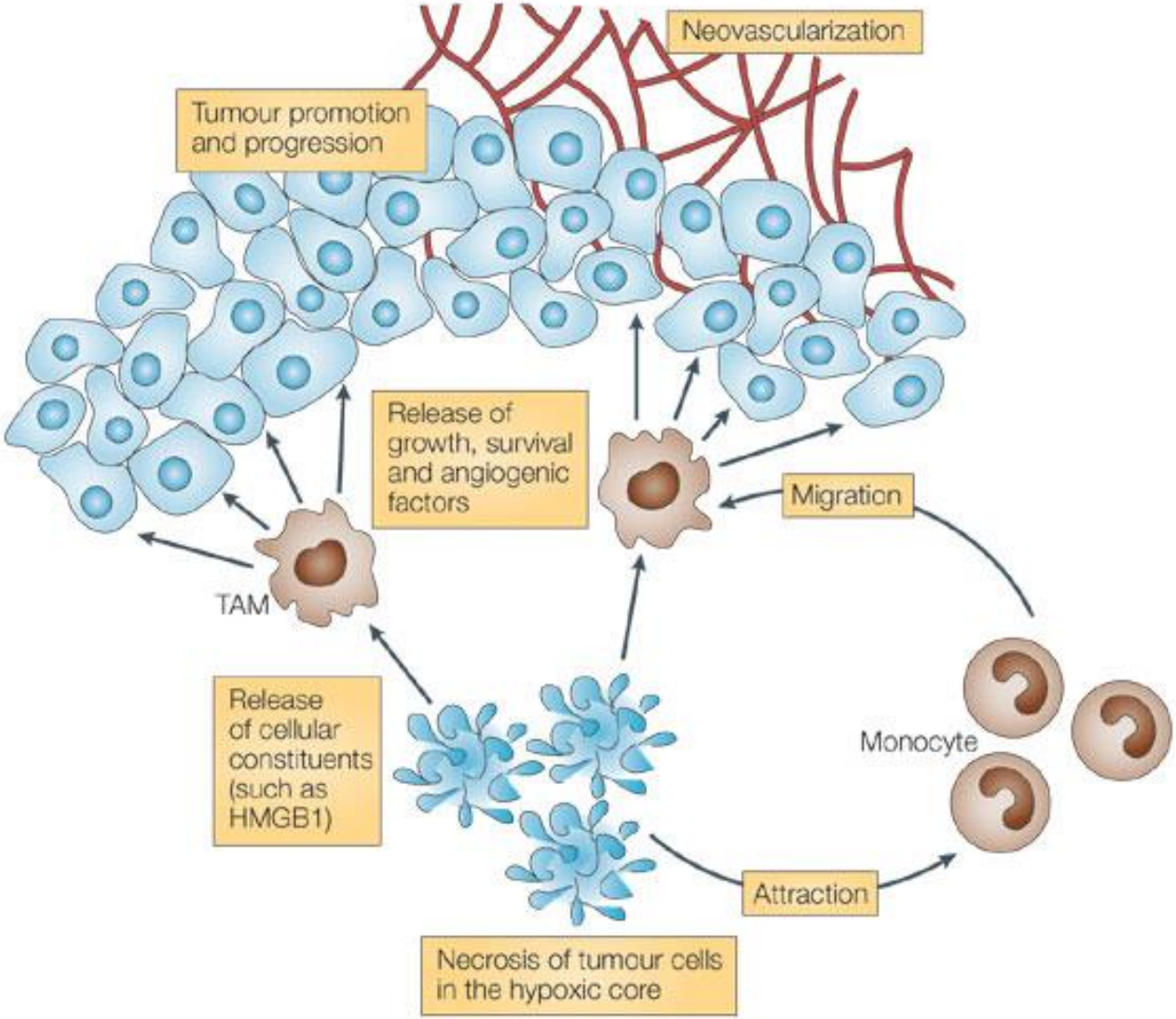

Figure 16.9 The Immune System, 3ed. (© Garland Science 2009)

**Tumor-associated antigen –** antigen expressed on tumor cells but also found on normal cells, often in smaller amounts.

# „Tumor escape”

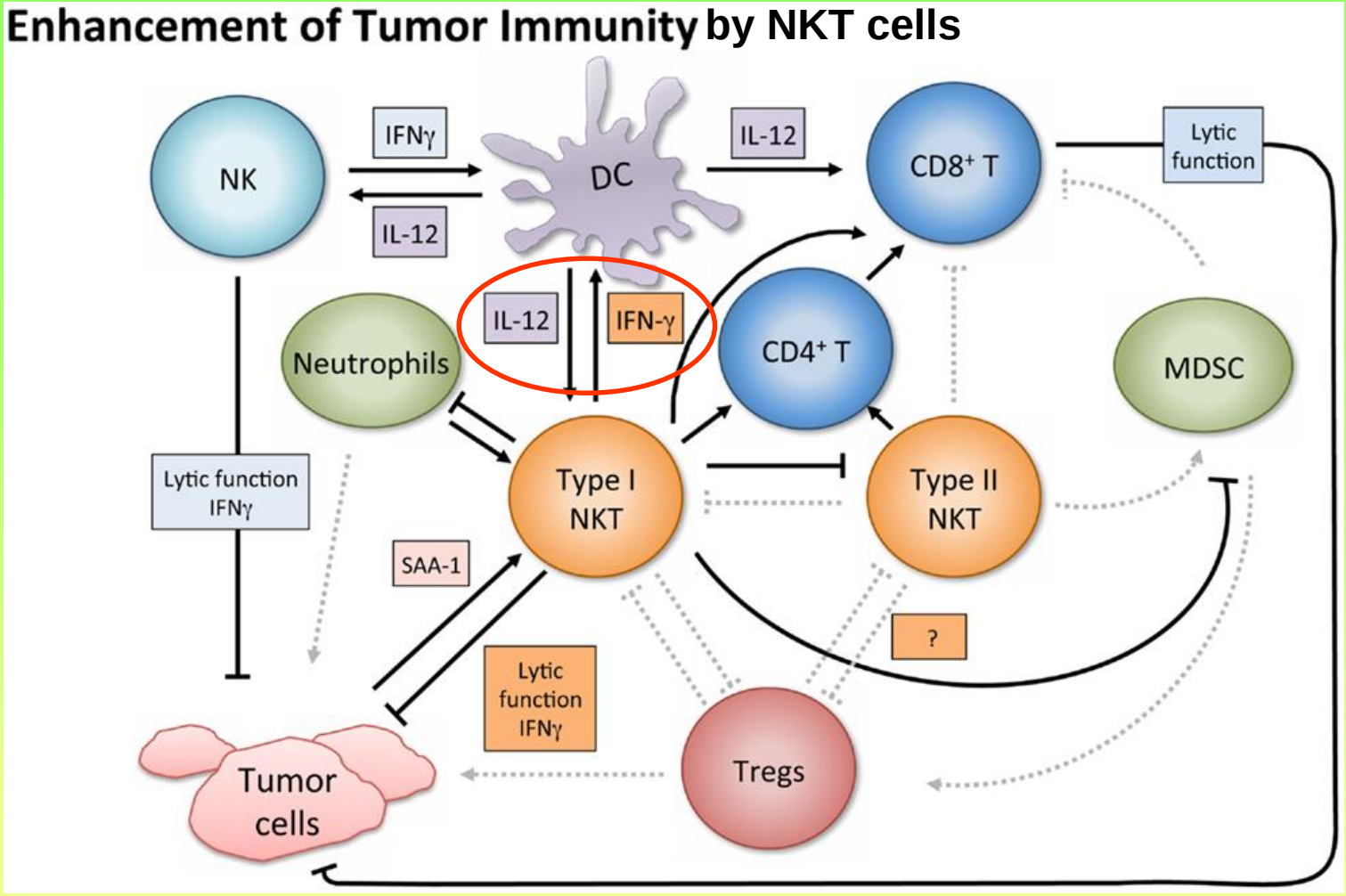
- Downregulation or owerexpression of MHC class I.
- Owerexpression of FcRs.
- Failed cytokine receptors.
- Masking and blocking glykoproteines.
- Production of tumor associated cytokines.
- Blocking cytokines produced by macrophages or dendritic cells.


CTL
CD8
High
class I MHC
Processed
tumor antigen
Killed
first
CTL
CD8
Moderate
class I MHC
Killed
later
Class I MHC
Tumor cell
Moderate
class I MHC
Escapes
Low
class I MHC
Escapes

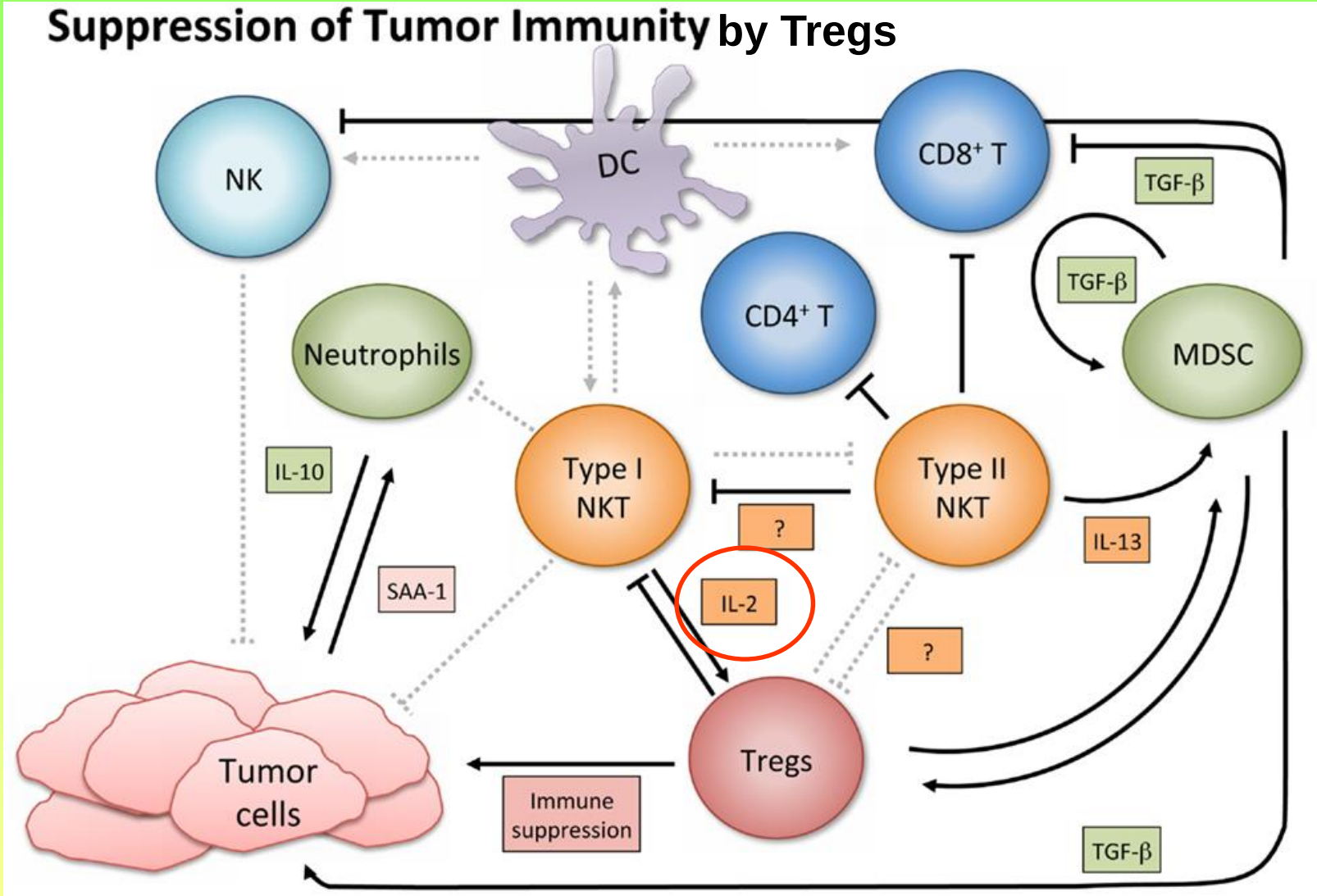




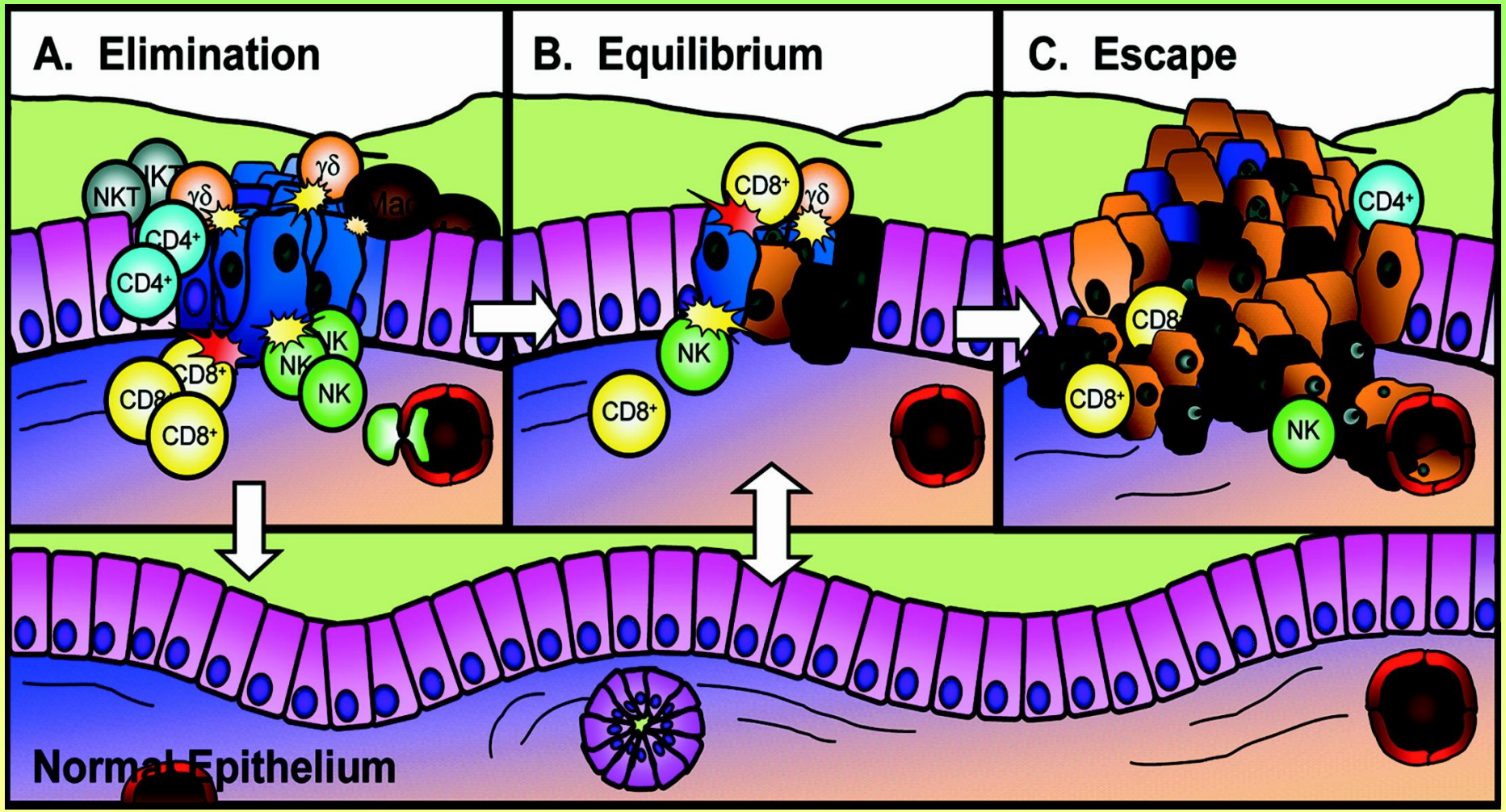
**Nature Reviews | Immunology**




Upon antigenic stimulation, type I NKT cells produce **IFN-γ** activate both CD8+ T cells and DCs. NKT cells specifically induce DC maturation by engaging the CD1d-TCR complex and CD40–CD40L interaction and upregulate costimulatory receptors of CD8+ T cells. Additionally, **IL-12** production by DCs stimulates NK, NKT, and other T cells to produce more IFN-γ and the two cytokines together significantly impact the activation of downstream effector populations.




Activated type I NKT cells can support immunosuppressive Tregs through **IL-2** production, and they are then suppressed by Tregs in a cell-contact-dependent manner. Treg cells can then suppress CD8+ and CD4+ T cells and NK cells as well at the same time.

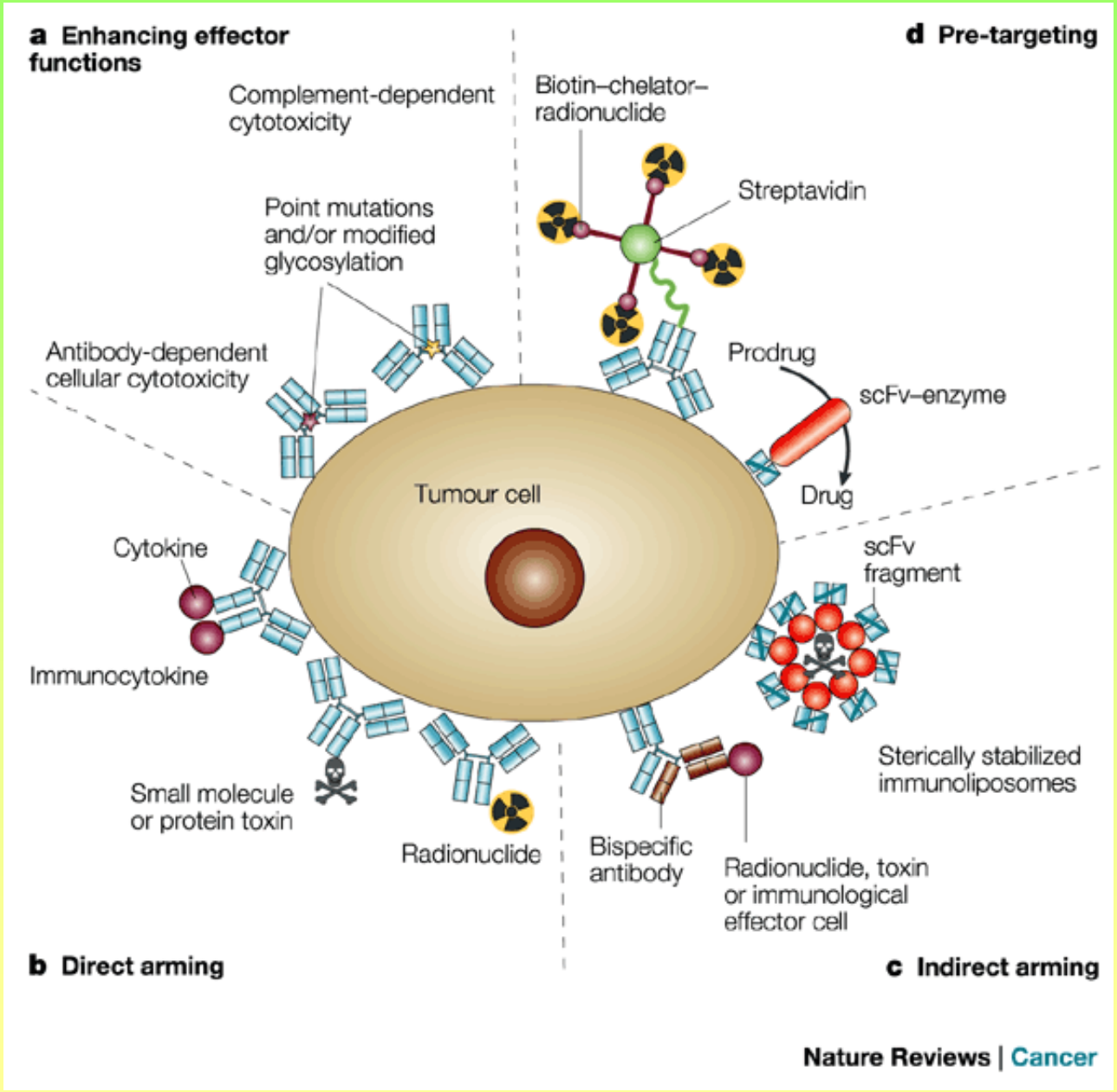

A. Elimination
B. Equilibrium
C. Escape
NKT
γδ
CD4+
CD8+
NK
Normal Epithelium

# Cancer immunotherapy

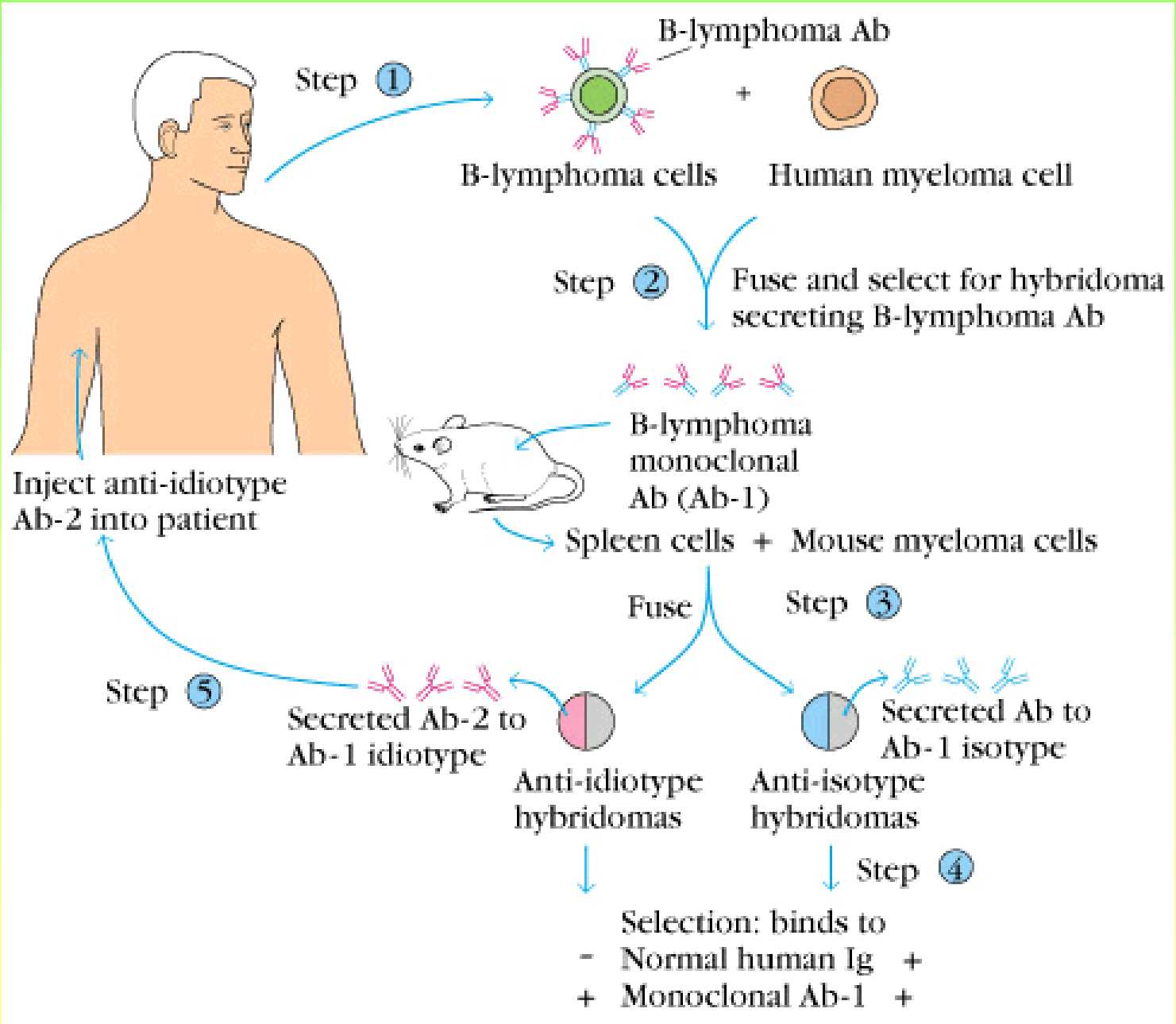
Complementary therapeutic tools after the surgical, chemotherapeutic and irradiation treatments:

- Therapeutic monoclonal antibodies
- Checkpoint inhibitors (PD-1/PDL-1)
- Immuno-modulation
- Cancer vaccines
- Oncolytic viruses

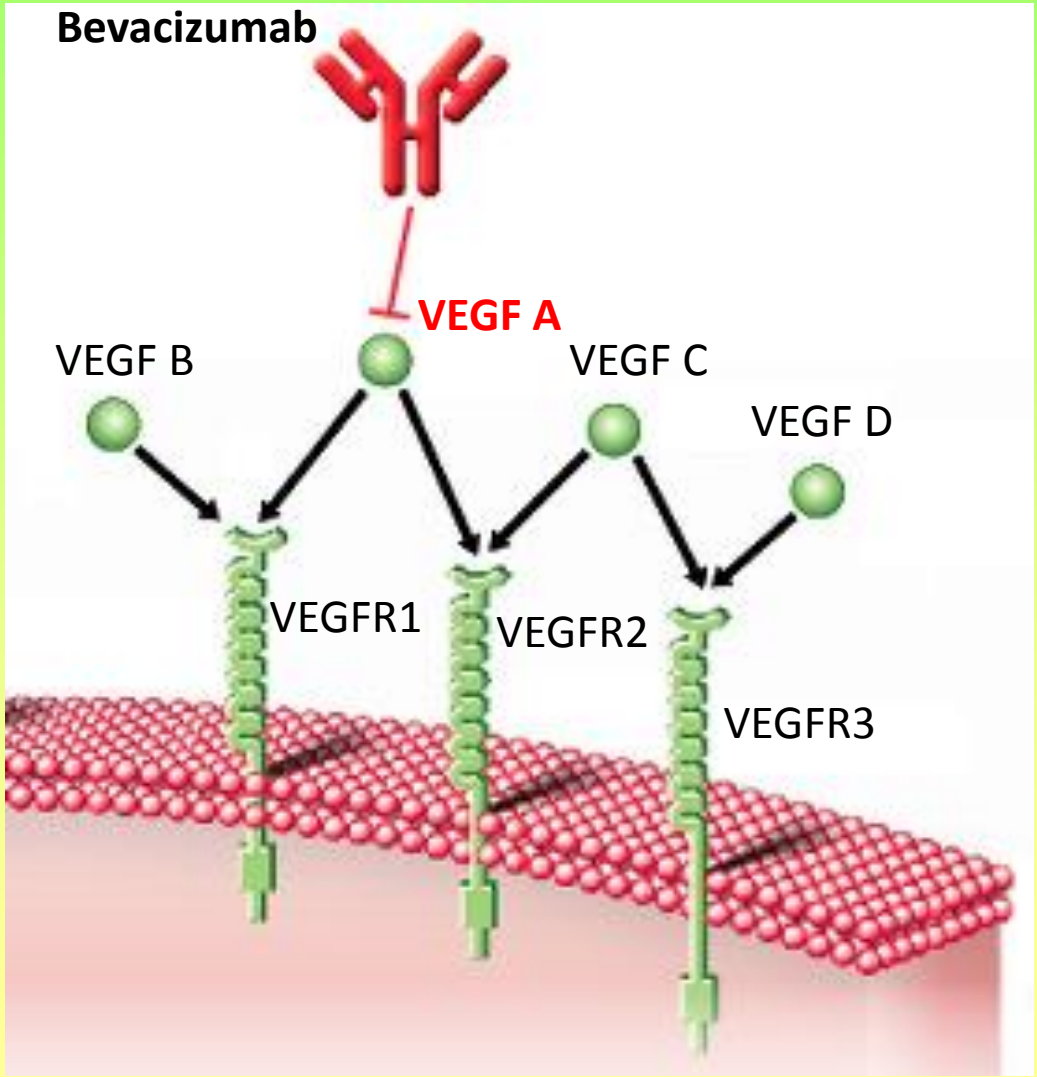
# Monoclonal antibodies for therapeutic use



# Anti-idiotype therapy of B cell malignant lymphomas



# Therapeutic monoclonal antibodies I.

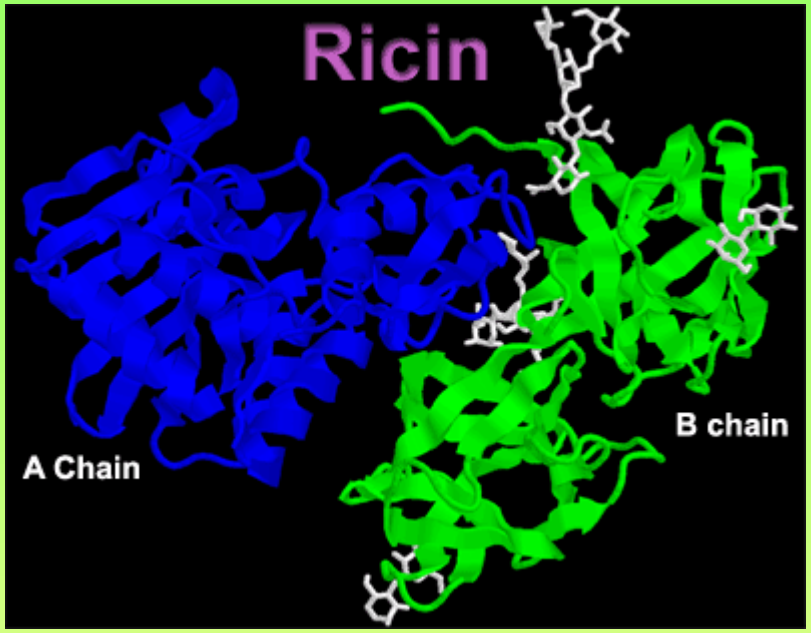


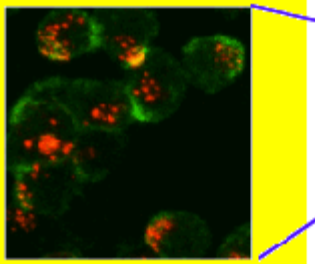

**Bevacizumab** (Avastin®)
Anti-VEGF A antibody

**Blockin of angioneogenesis**

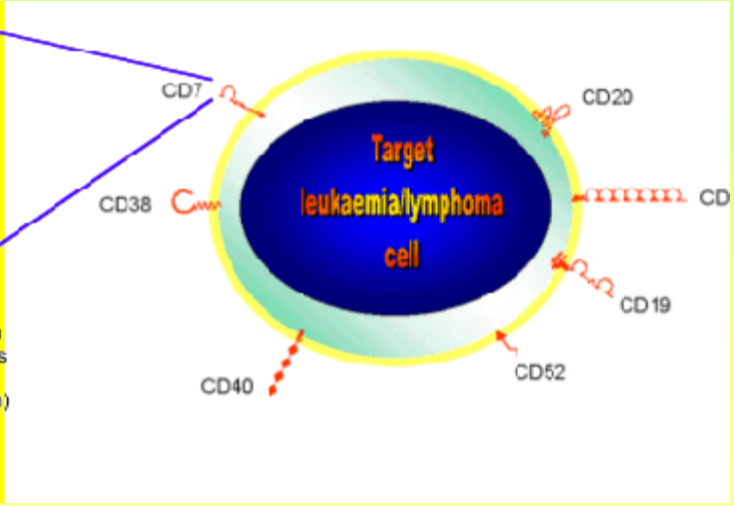
Applicable in some solid tumors:

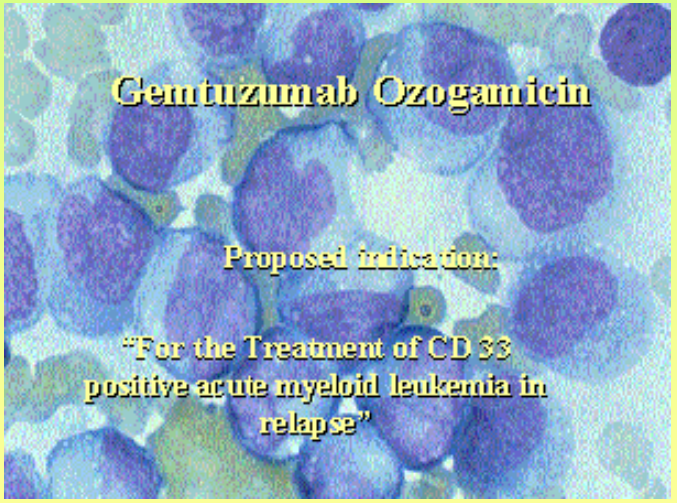
- Colon cancer
- Lung cancer
- Ovarian cancer
- Kidney cancer
- Glioblastoma

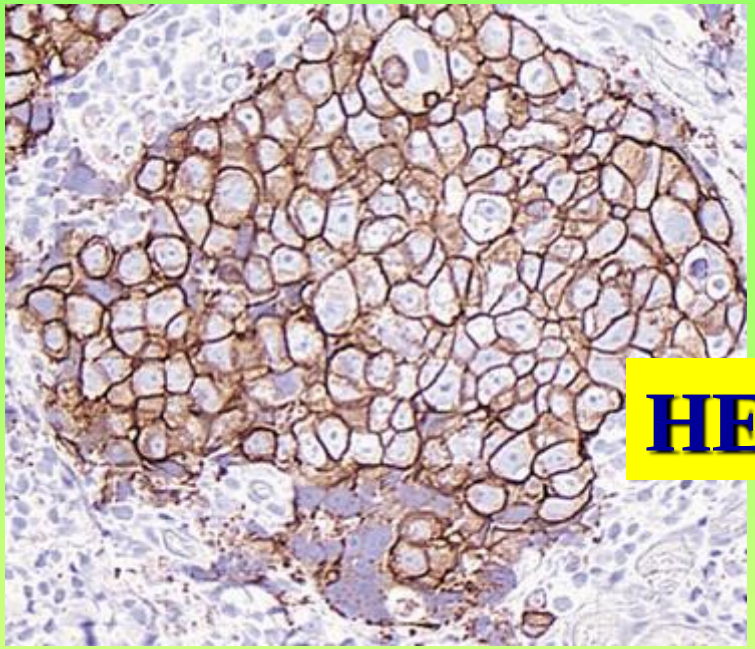


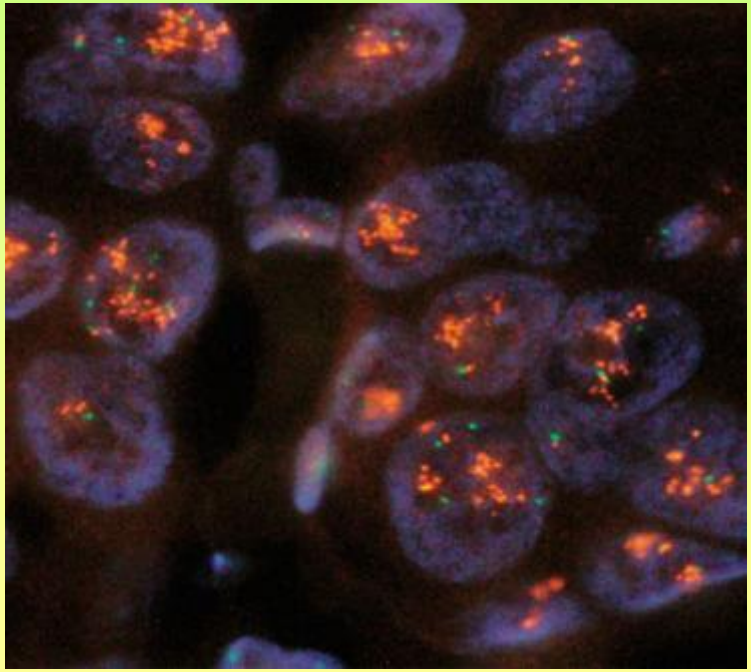
Confocal microscope image showing the internalisation of an anti-CD7 saporin immunctoxin by human T- leukaemia cells into intracellular vesicles (green stain) in relation to the trans-golgi region (red stain)

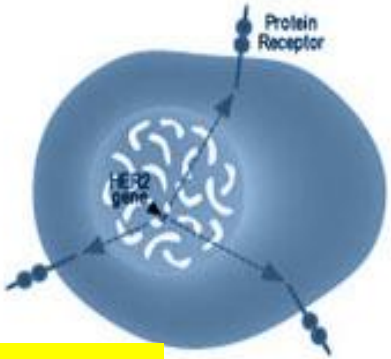




# HER-2/neu



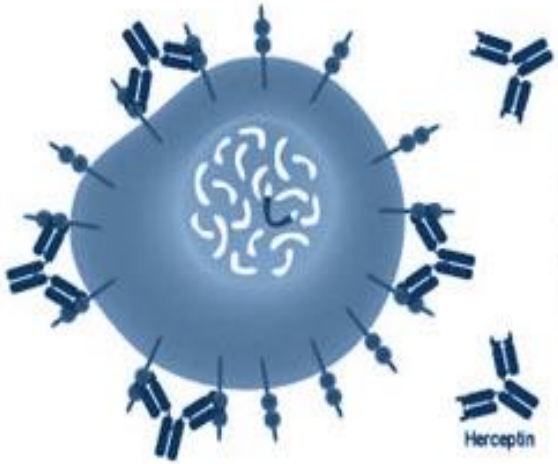



## Normal Cell

In normal breast tissue cells, the HER2 gene produces a protein receptor on the cell surface. These growth factor-like receptors are thought to play a role in normal cell growth by signaling the cell to divide and multiply.

## HER2 Overexpressing Cancer Cell

Cancerous breast tissue cells that overexpress (or overproduce) the HER2 gene produce extra protein receptors on the cell surface. The higher density of receptors triggers the cell to divide and multiply at an accelerated rate, thus contributing to tumor growth. Approximately 25-30% of all women with metastatic breast cancer overexpress the HER2 protein.



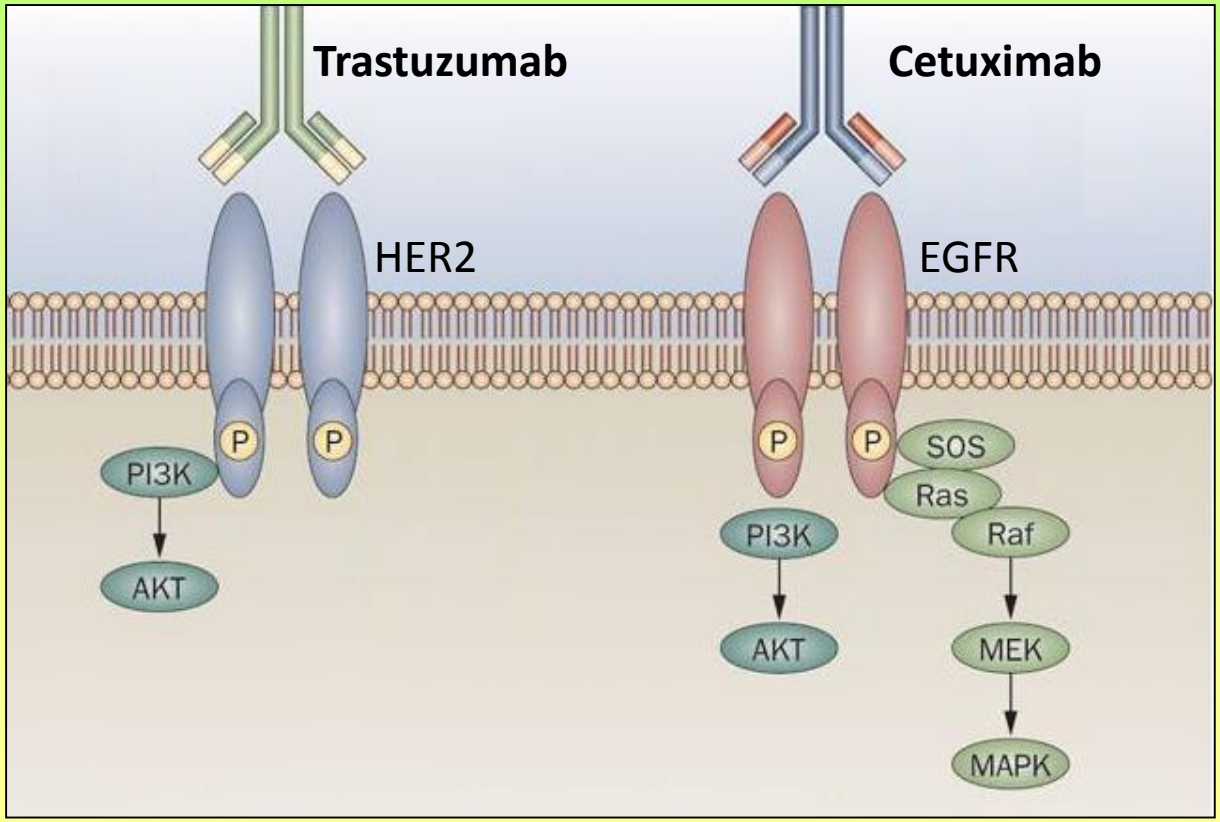

## Herceptin® (Trastuzumab)

It is thought that Herceptin (a HER2 antibody) binds to numerous HER2 receptor sites found on the cell surface, blocking the receptor sites and possibly preventing further growth by interrupting the growth signal. As a result, the HER2 antibody may slow progression of the disease.

# Therapeutic monoclonal antibodies II.

**EGFR** blockers:

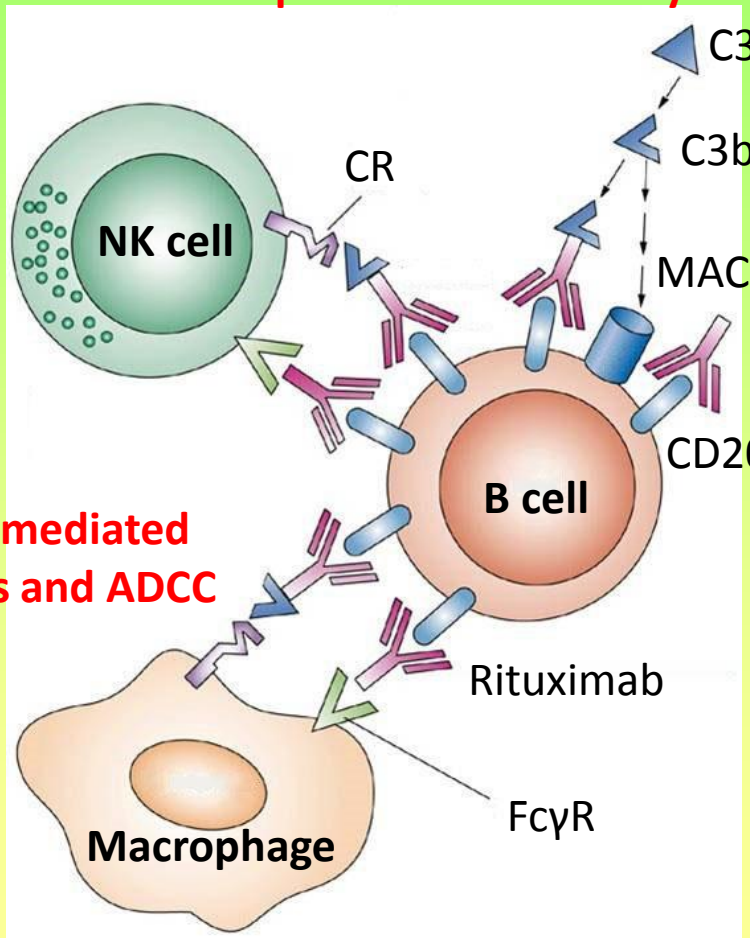
- **Trastuzumab** (Herceptin®): anti-EGFR2 (HER2) → **HER2 positive breast cancer and gastric cancer**
- **Cetuximab** (Erbitux®) → colon cancer, lung cancer, head and neck cancers



# Therapeutic monoclonal antibodies III.

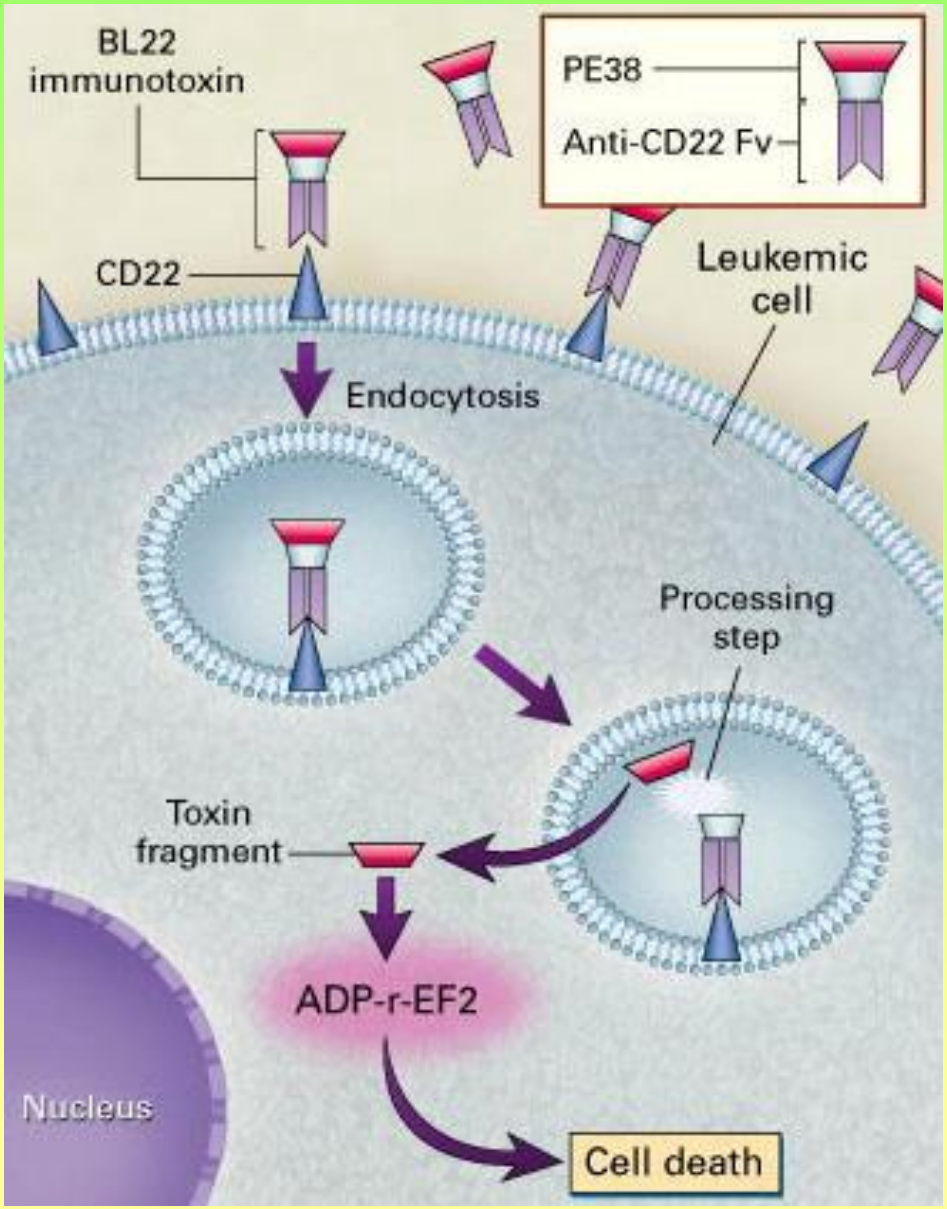
**Antibodies against trageted cells**:

- **Rituximab** (MabThera®): anti-CD20 → **B cell deplation** (E.g. B cell lymphomas, autoimmune diseases
- **Alemtuzumab:** anti-CD52 → CLL, sclerosis multiplex




**Mechanism of action of the Rituximab**

# Therapeutic monoclonal antibodies IV.




Treatment of hairy cell leukemia with BL22 immunotoxin therapy

# Therapeutic monoclonal antibodies V.

## T cell activating antibodies

Nivolumab:
Anti-PD1 antibody

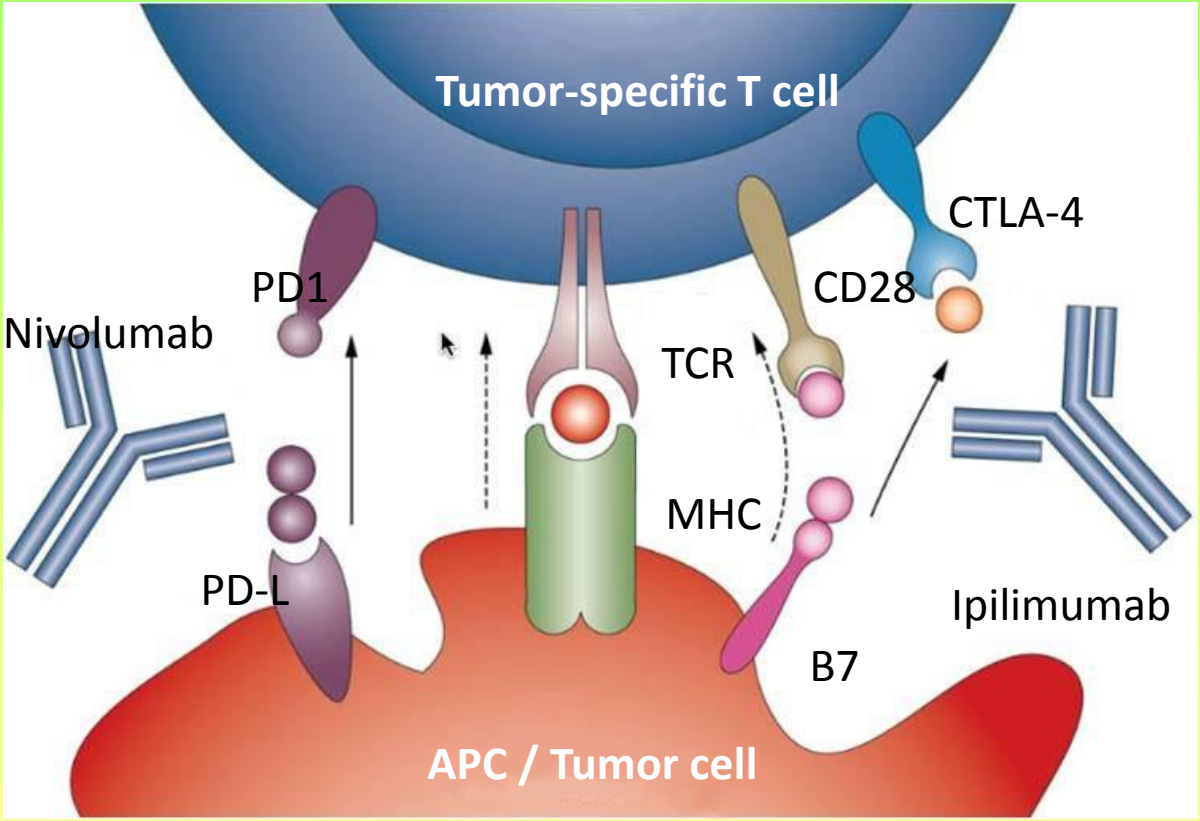
Ipilimumab:
Anti-CTLA-4 antibody

↓

Neutralization of the blocking effects of PD1 and CTLA-4

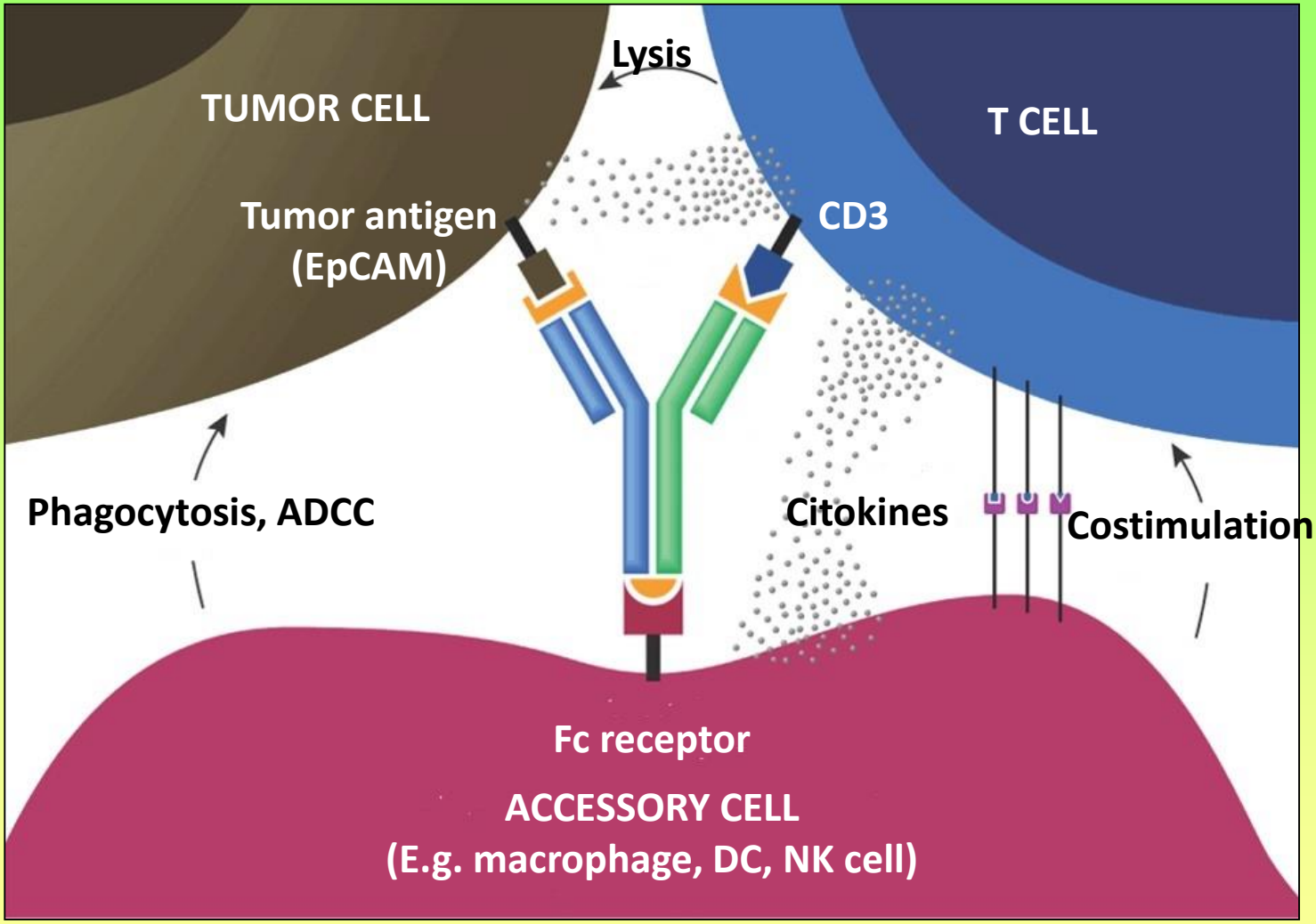
↓

**T cell tolerance is decreasing**



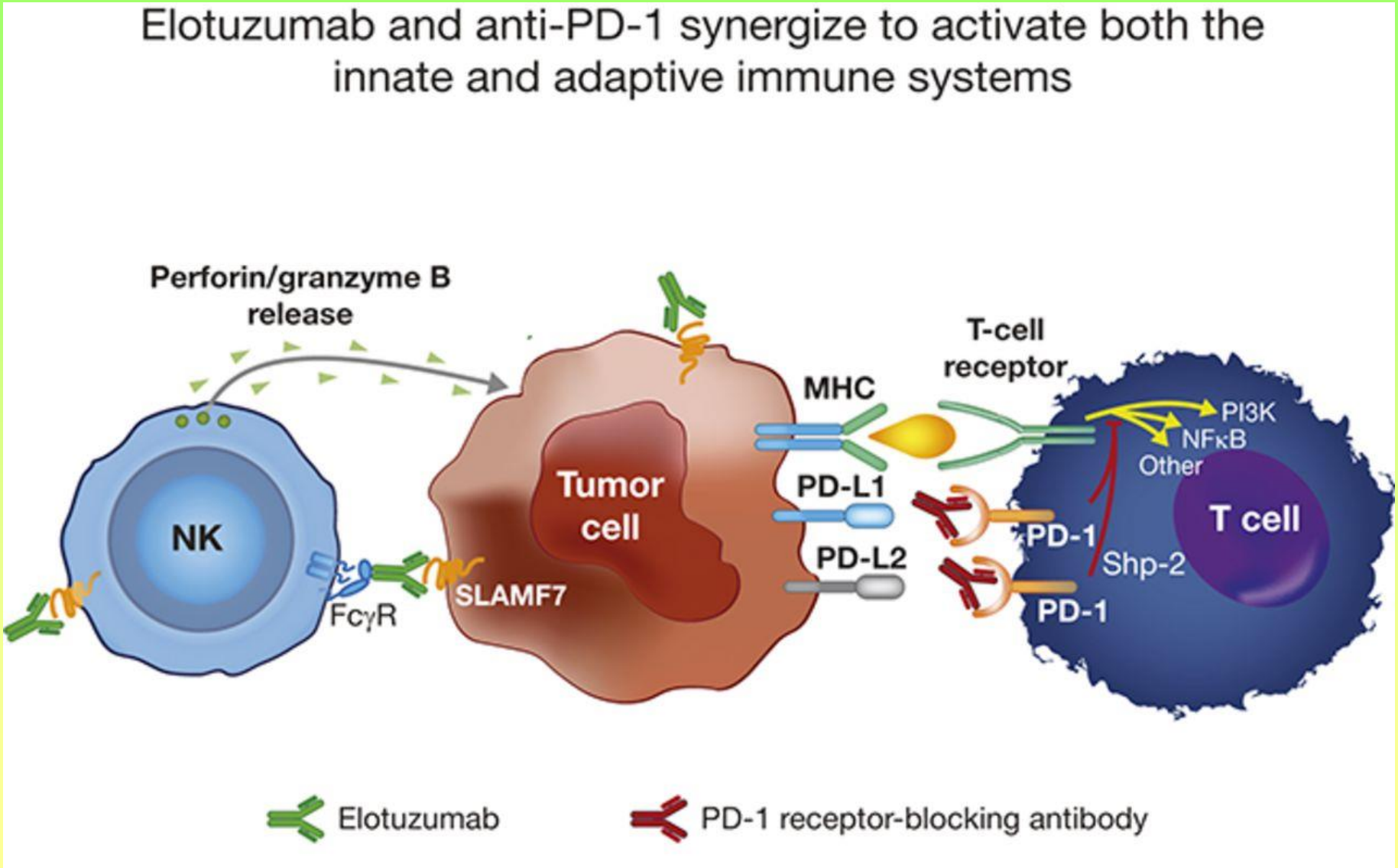

Therapeutic tool in melanoma malignum. (T cells are able to kill tumor cells without inhibition.
**Inhibition of inhibitors = activation!**)

# Bispecific therapeutic monoclonal antibodies

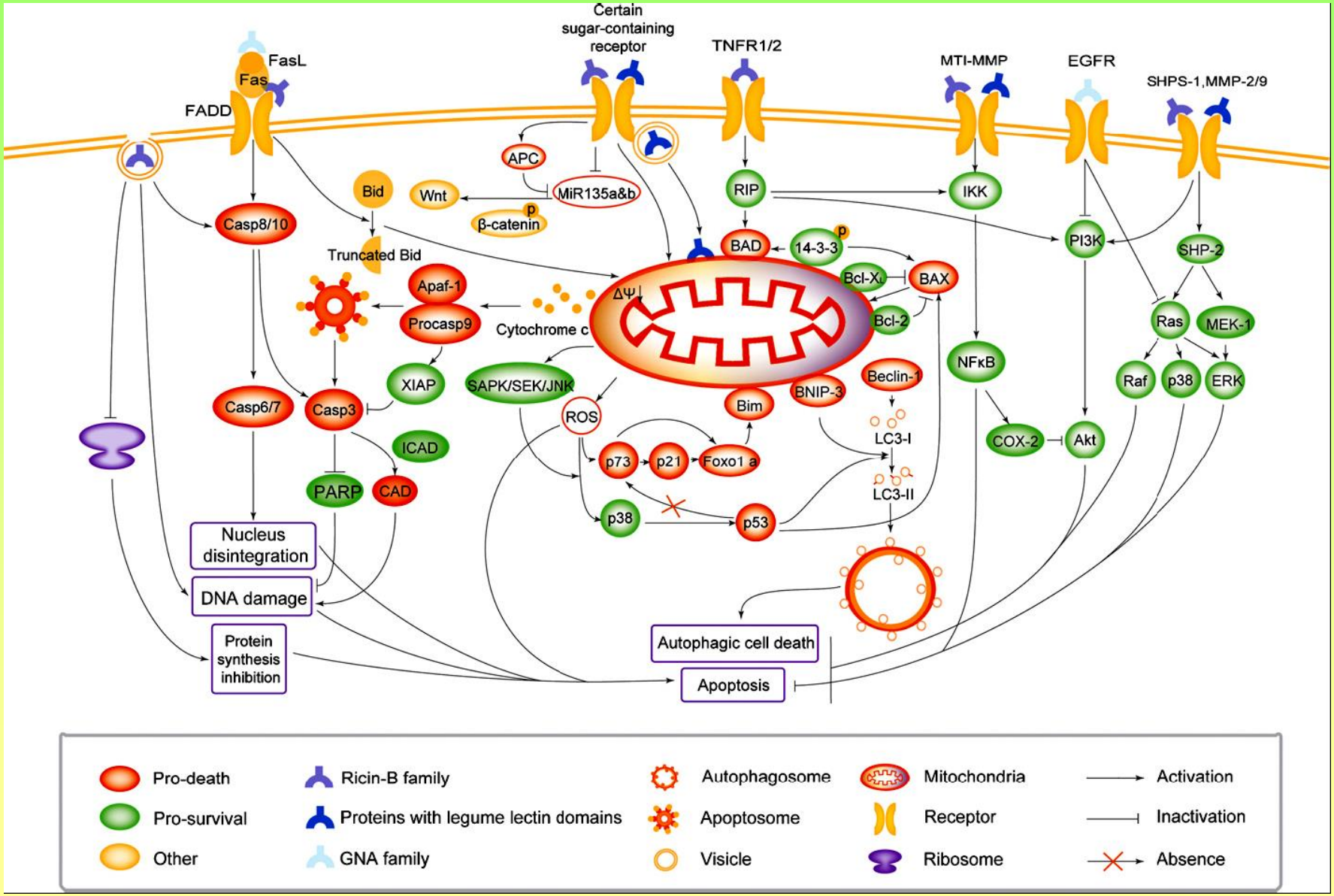



Mechanism of action of Catumaxomab (the first approved bispecific and és trifuntional antibody). (EpCAM: Epithelial cell adhesion molecule)

# Checkpoint inhibitors



# Plant lectins induce cancer cell death via targeting programmed cell death (PCD) signaling network.



# Mistletoe

Mistletoe is a semiparasitic plant, holds interest as a potential anticancer drug because extracts derived from it have been shown to kill cancer cells *in vitro,* and stimulates immune system both *in vitro* and *in vivo*.

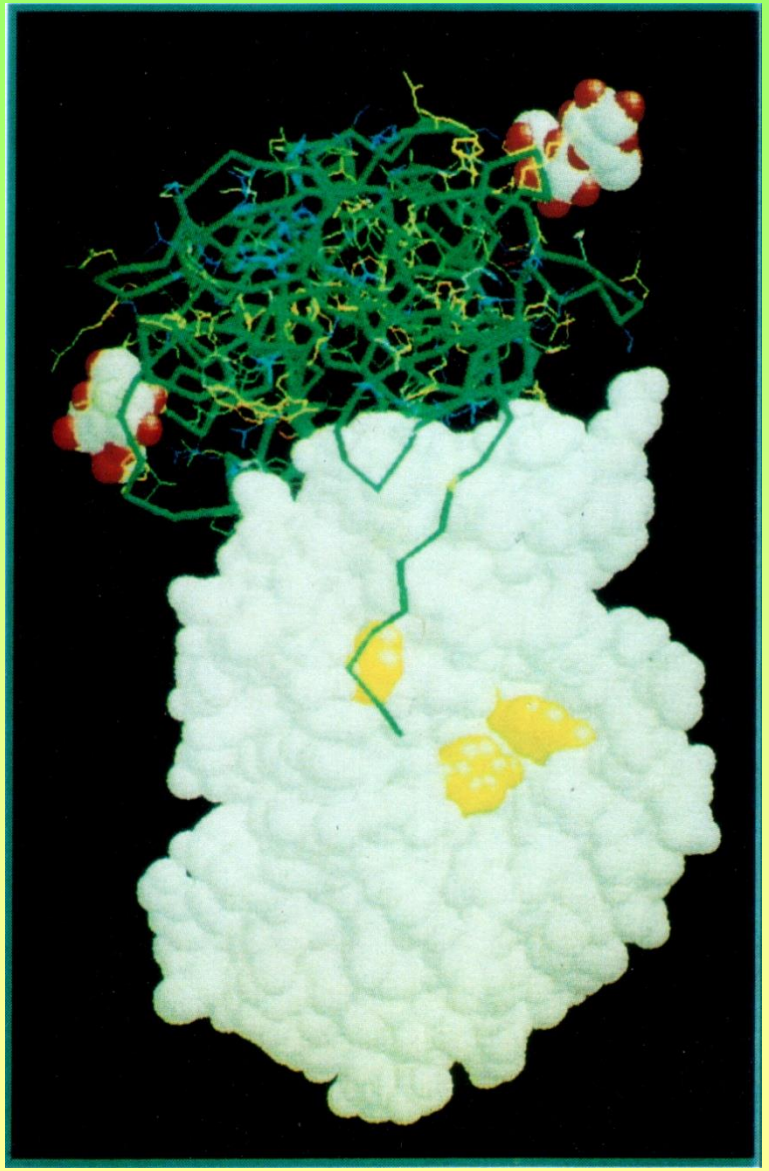
# Mistletoe lectin as immunomodulator

**Two chains of the *Viscum Album* Agglutinin-I (VAA-I) :**

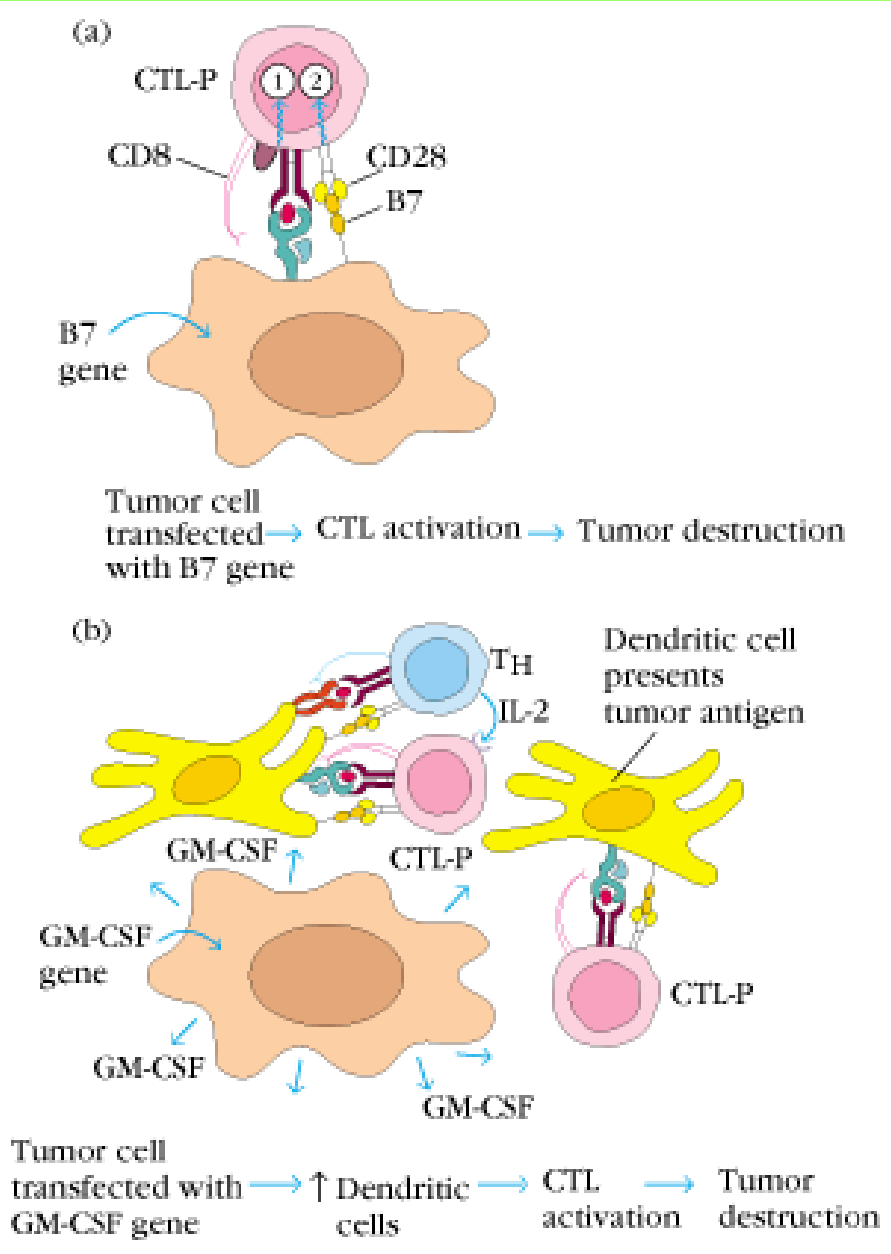
• „A chain” (29 kD) strong ribosoma inactivator by the N-glikosidase activity.

• Sugar binding „B cahian” (34 kD) is responsible for the **imunomodulant activity.**

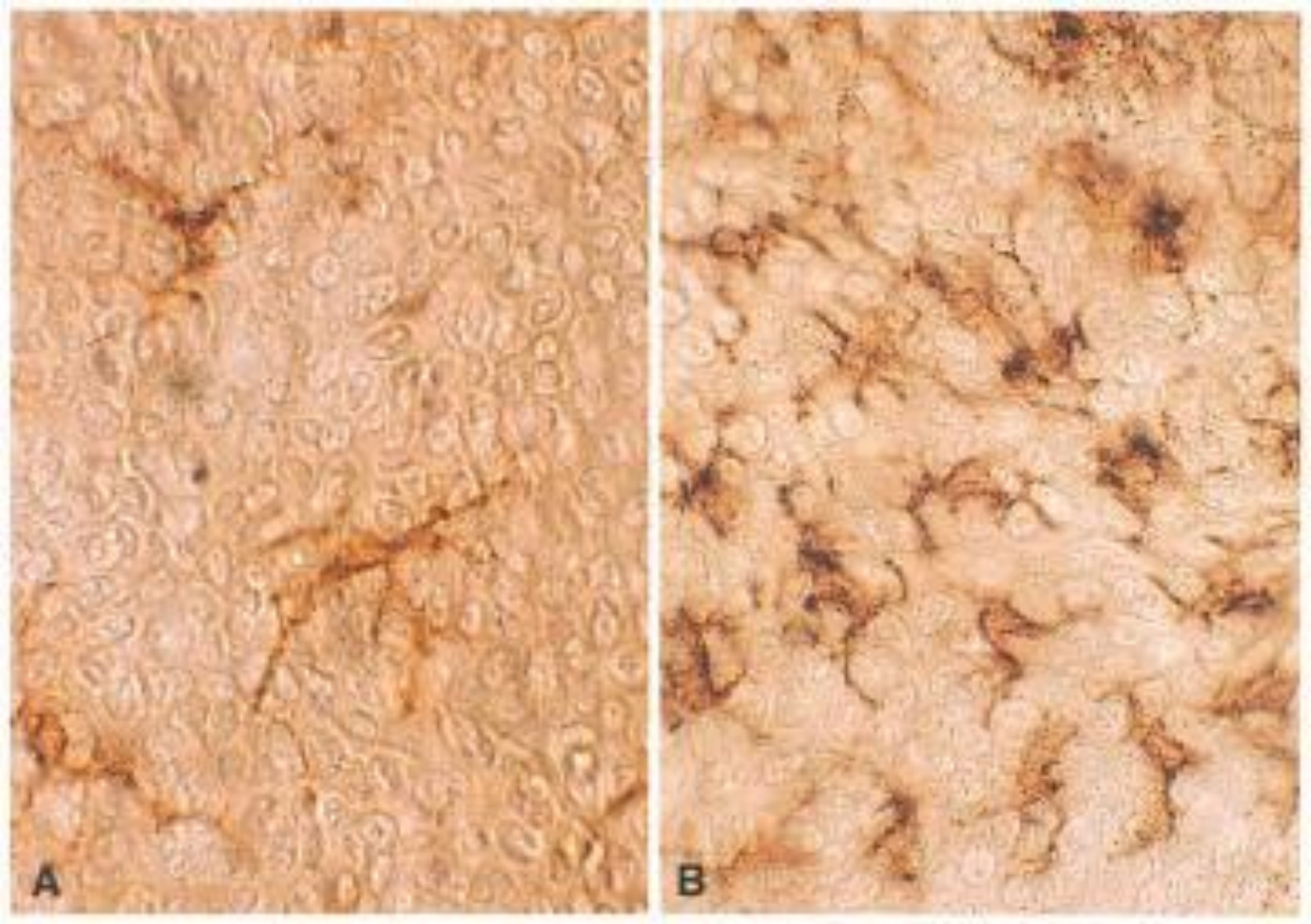
**Enhance the T cell and NK cell maturation and activation in dose dependent manner**.



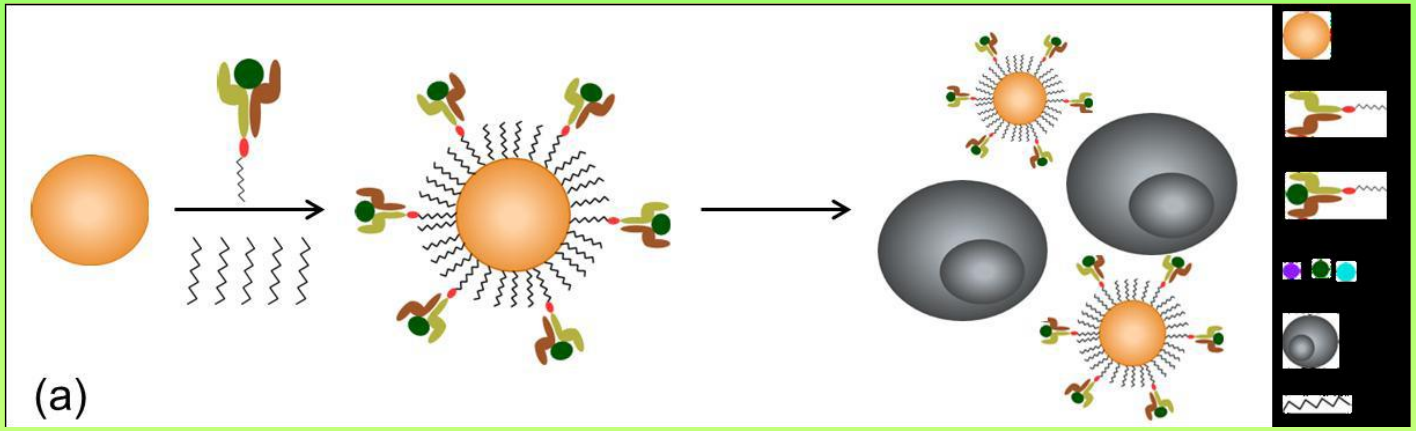
# Development of cancer vaccines



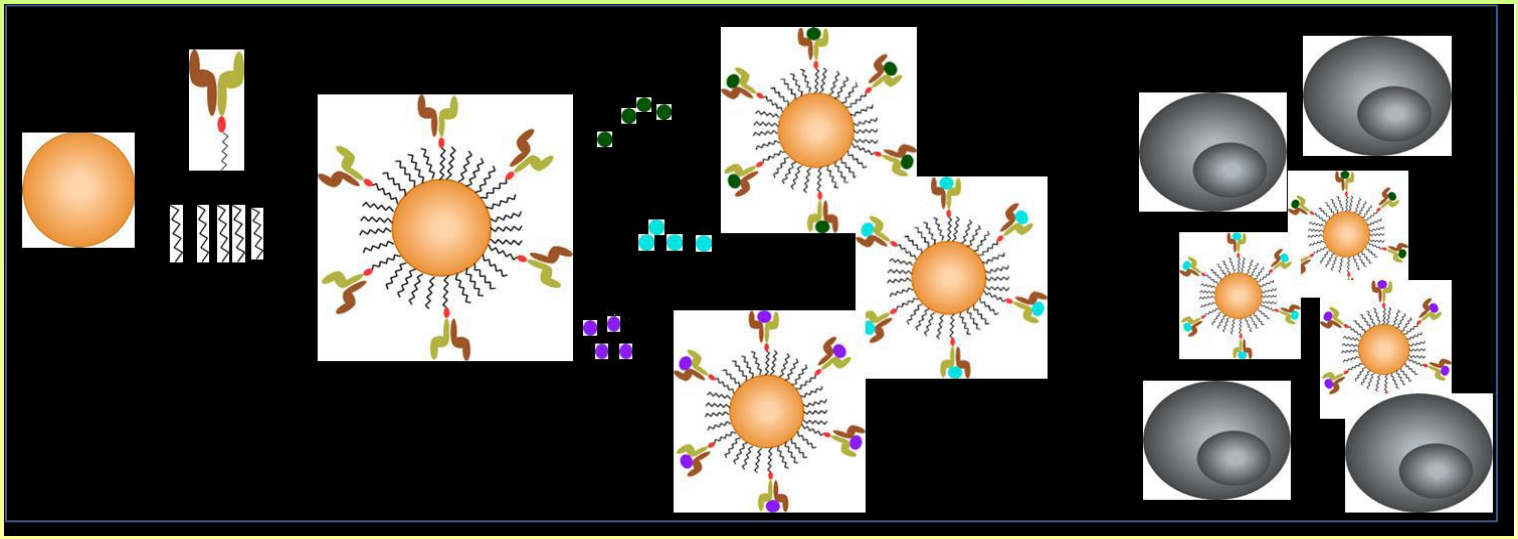
# Recruitment of dendritic cells by DNA vaccine of GM-CSF



# Nanoparticles for therapeutic use



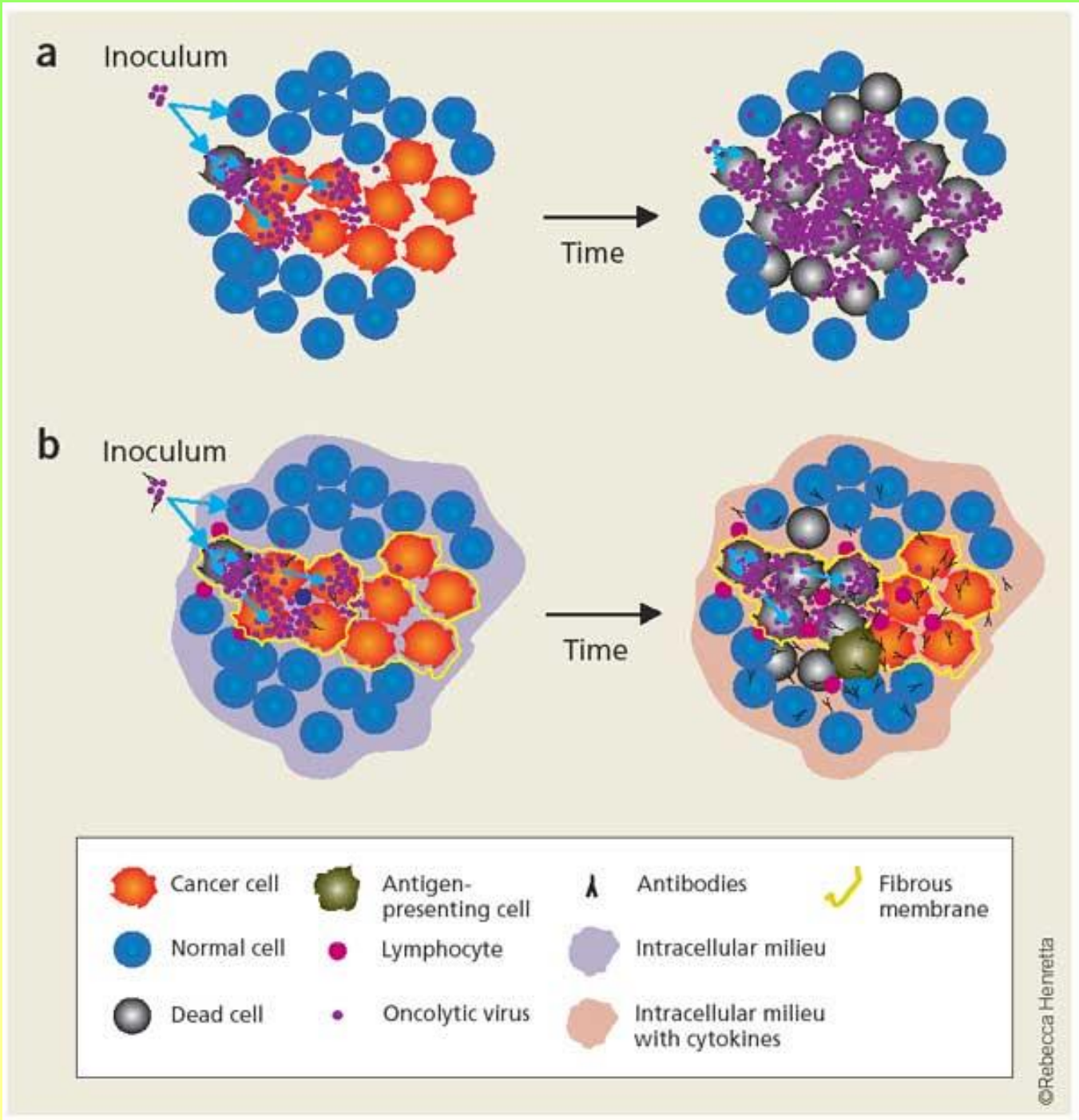

**(a) Gold Nanoparticles are functionalized with PEG + MHC-peptide complexes. The functional NPs are designed to interact with T-Cells.**



**(b) NPs are functionalized with PEG+MHC complex, free of peptides. Various peptides are then loaded on the MHC NPs. The functional NPs are designed to interact with T-Cells**

# Immuntherapy with oncolytic viruses

- An ***oncolytic virus*** is a virus that preferentially infects and lyses cancer cells; these have obvious functions for cancer therapy, both by **direct destruction** of the tumor cells, and, if **modified, as vectors** enabling genes expressing anticancer proteins to be delivered specifically to the tumor site.
- Most current oncolytic viruses are **engineered** for tumour selectivity, though there are a few naturally occurring ones such as the *Seneca Valley* virus .


a
Inoculum
Time
b
Inoculum
Time
Cancer cell
Antigen-presenting cell
Antibodies
Fibrous membrane
Normal cell
Lymphocyte
Intracellular milieu
Dead cell
Oncolytic virus
Intracellular milieu with cytokines
©Rebecca Henretta

# Difficulties of the oncolytic virus therapies

Viral agents administered intarvenously can be particularly effective against metastatic cancers, which are especially difficult to treat conventionally.

However, blood-borne viruses can be deactivated by antibodies and cleared from the blood stream quickly e.g. by Kupfer cells in the liver, which are responsible for adenovirus clearance.

# Immunotherapy of tumors

| | | |
|---|---|---|
| active | non-specific | BCG, *Propionibacterium acnes*, levamisole, cytokine genes, *etc*. |
| | specific | killed tumor cells or their extract, recombinant antigens, idiotype, co-stimulatory molecule genes, *etc*. |
| passive | nonspecific | LAK cells, cytokines |
| | specific | antibodies alone or coupled to drugs, pro-drug toxins or radioisotope; bispecific antibodies; T-cells |
| | combined | LAK cells and bispecific antibody |

* BCG: Bacillus Calmette Geurin is a bovine strain of *Mycobacterium tuberculosis*.

# Non-specific active immunotherapy: biological response modifiers (BRMs)

| type of BRM | examples | major effect |
|---|---|---|
| **bacterial product** | BCG, *P. acnes*, muramyl di-peptide, trehalose dimycolate | activate macrophages and NK cells (via cytokines) |
| **synthetic molecules** | pyran, poly I:C, pyrimidines | induce interferon production |
| **cytokines** | interferon-alpha, -beta, -gamma, IL-2, TNF | activate macrophages and NK cells |

# Cytokine therapy of tumors

| cytokine | tumor type and result | antitumor mechanism(s) |
|---|---|---|
| **IFN-alpha, beta** | remission of hairy cell leukemia, weak effect on some carcinomas | increased expression of class I MHC, possible cytostatic anti-tumor effect, |
| **IFN-gamma** | remission of peritoneal carcinoma of ovary: ineffective systemically | increased MHC antigens; macrophage, Tc and NK cell activation |
| **IL-2** | remission in renal carcinoma and melanoma | T-cell proliferation and activation, NK cells activation |
| **TNF-alpha** | can reduce malignant ascites | macrophage and lymphocyte activation |

# COMBINATORIAL IMMUNOTHERAPY

## A Promising New Way To Kill Cancer