

# Teaching unit 14

## Immunotherapy of malignant tumors

# Tumor therapy

- **Chemotherapy**
- The hypothesis of fractional cytotoxicity assumes that a certain concentration of cytostatics, applied for a certain period of time, kills a constant fraction of the population of tumor cells. In this way, each subsequent therapeutic cycle kills the same fraction of remaining cells.
- For example, if the cytotoxic effect is 99% per cycle, the tumor cell population of  $10^{11}$  will be reduced to less than one cell in six cycles:  $[10^{11} \text{ cells}] \cdot [0.01]^6 < 1$ .

# Tumor therapy

- Resistance
- It is generally known that the emergence of drug resistance (cytostatic) is a consequence of random mutations in the population of tumor cells.
- The possibility of de novo emergence of resistance in any population of tumor cells increases with the number of cells and the number of cell divisions.
- Indiscriminateness (non-selectivity)
- Toxicity

# Challenge

How to create a tumor-specific therapy based on the biological characteristics of the tumor?

# Biotherapy

Biotherapy represents the first attempt to make cancer therapy specific to malignant cells

# Biotherapy

- **Biotherapy** involves the use of agents of biological origin or agents that modify the biological response
- **Synonyms:** biological therapy, immunotherapy, therapy with biological response modifiers
- **Immunotherapy** is a form of biotherapy that uses and modifies the immune system, the cells and molecules of the immune system involved in the immune response, in the fight against disease.

# Immunotherapy - the beginnings

- **William Bradley Coley** (William Bradley Coley), 19th century, American surgeon who established the connection between infection and spontaneous tumor regression.
- Sarcoma-postoperative erysipelas-regression (Coley, 1893; Coley, 1911).
- Used bacterial toxins in tumor therapy.

# Immunotherapy - the beginnings

Three key observations that enabled the emergence of immunotherapy:

- Spontaneous remission of some tumors
- Increased incidence of tumors in immunosuppressed persons
- Presence of lymphocytic infiltrates in tumors



# Immunotherapy - the beginnings

- A common name for agents used in biotherapy is biological response modifiers.
- Today, large amounts of biological response modifiers can be made in the laboratory and used in the therapy of many diseases, such as malignant tumors, chronic HCV infection, rheumatoid arthritis...

# Immunotherapy of malignant tumors

- non-specific immunotherapy
- 1959, Biozzie: Bacille Calmette-Guérin (BCG), a nonspecific activator of the mononuclear-phagocytic system, slows tumor growth
- costimulators

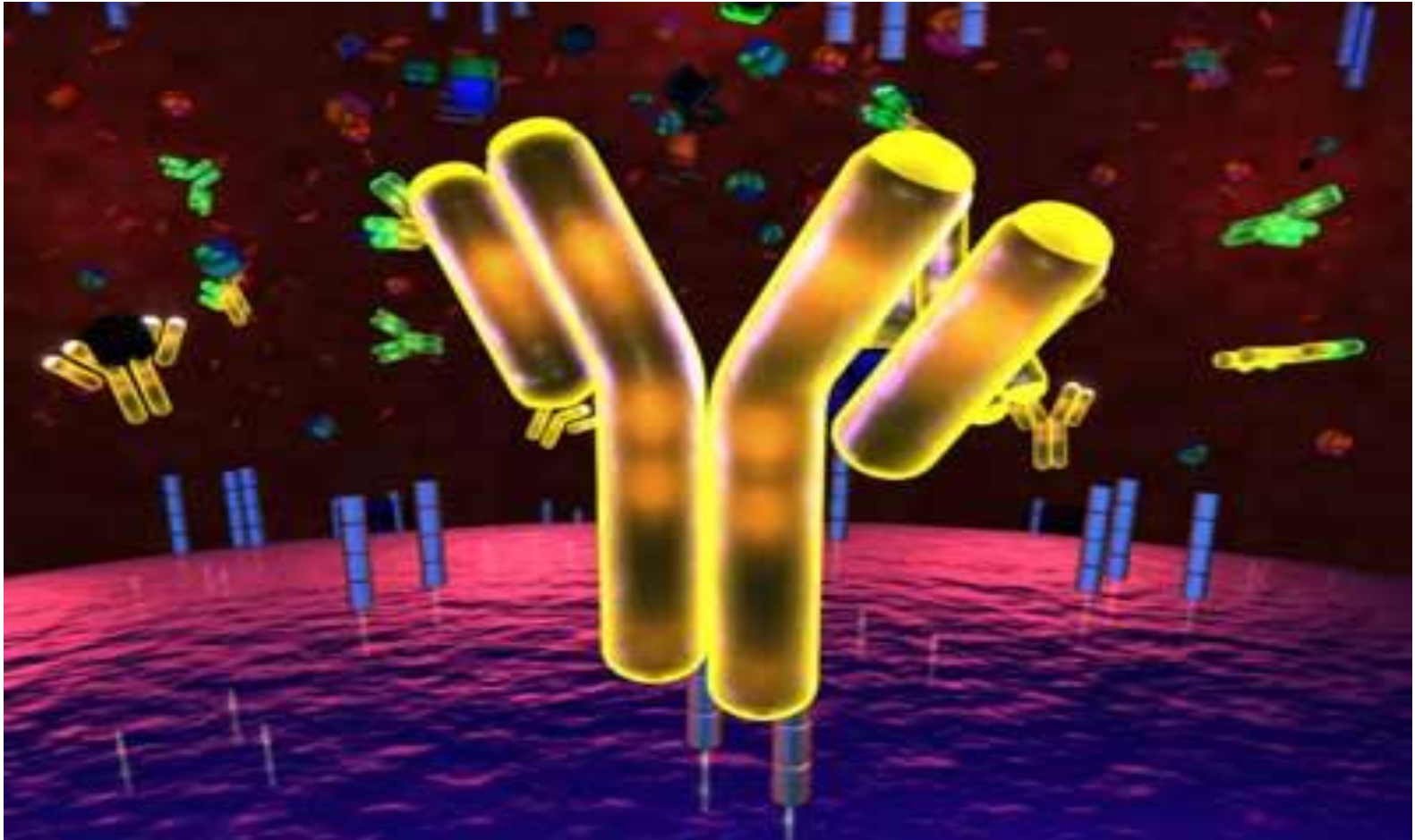
# Cytokines

- IFN- antiproliferative and immunomodulatory is applied:
- As monotherapy in low, medium or high doses.
- In combination with other cytokines (most often IL-2) or as part of chemotherapy

# Cytokines

- IL-2 was approved in 1992 for the treatment of metastatic kidney cancer, and in 1998 for the treatment of metastatic melanoma.
- It is used for the production of LAK and TIL cells, but also independently, most often in combination with IFN, and recently in numerous tumors in combination with IL-12, passive mAb immunotherapy and chemotherapy
- In high doses of IL-2 - marked toxicity
- Application is limited to sc administration.

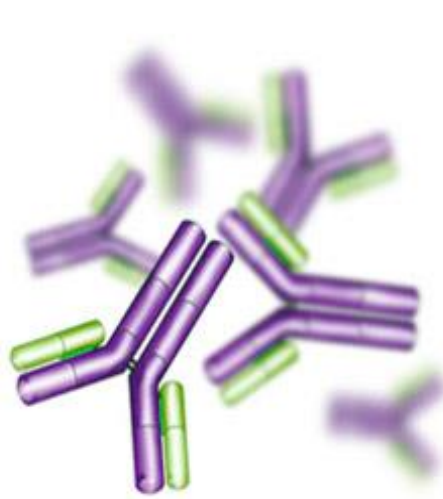
# Antibodies



- polyclonal antibodies
- monoclonal antibodies



*Cesar Milstein*



*George Kohler*



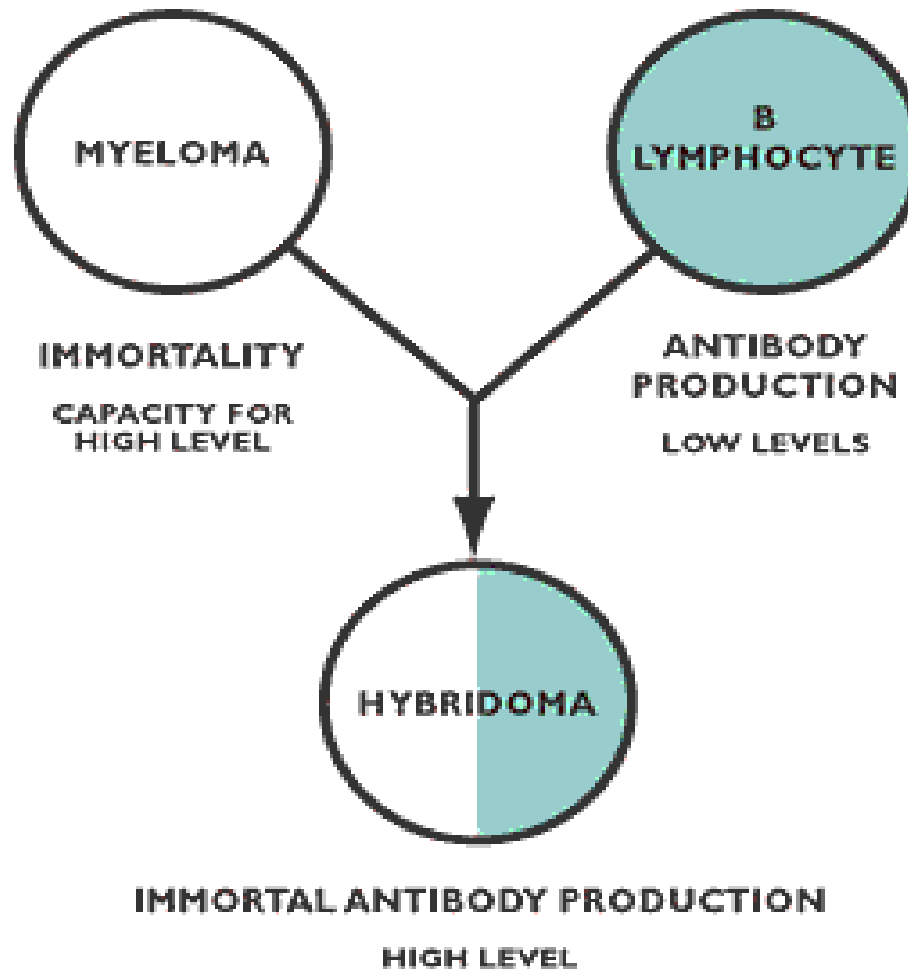
*C. Milstein and G. Kohler they are the first, in year 1975, introduced a technique for the production of monoclonal antibodies  
Year 1984 they won the Nobel Prize*

Due to the fact that clone of B lymphocytes that secretes antibodies of certain specificity has a limited half-life, *C. Milstein* and *G. Kohler* developed a method for the immortalization of these cells.

This method is based on **fusion** (merging) B lymphocytes with malignant cells myeloma in which they produce **hybrid cells that produces specific antibodies** (characteristic of B lymphocytes) and **are immortal and constantly multiply** (characteristic of malignant cells).

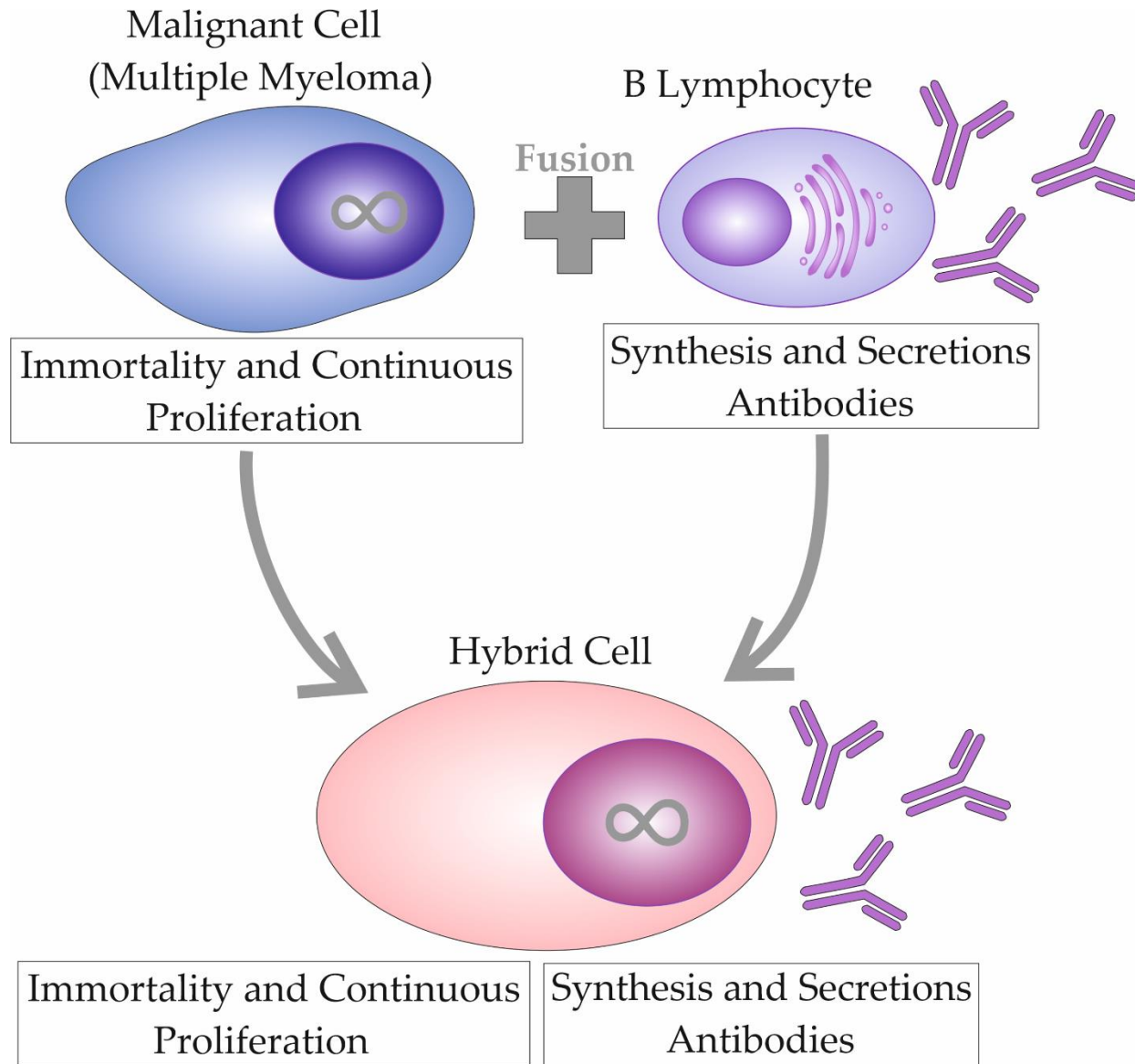
Georges Kohler & Cesar Milsterin 1975.

## Hybridization

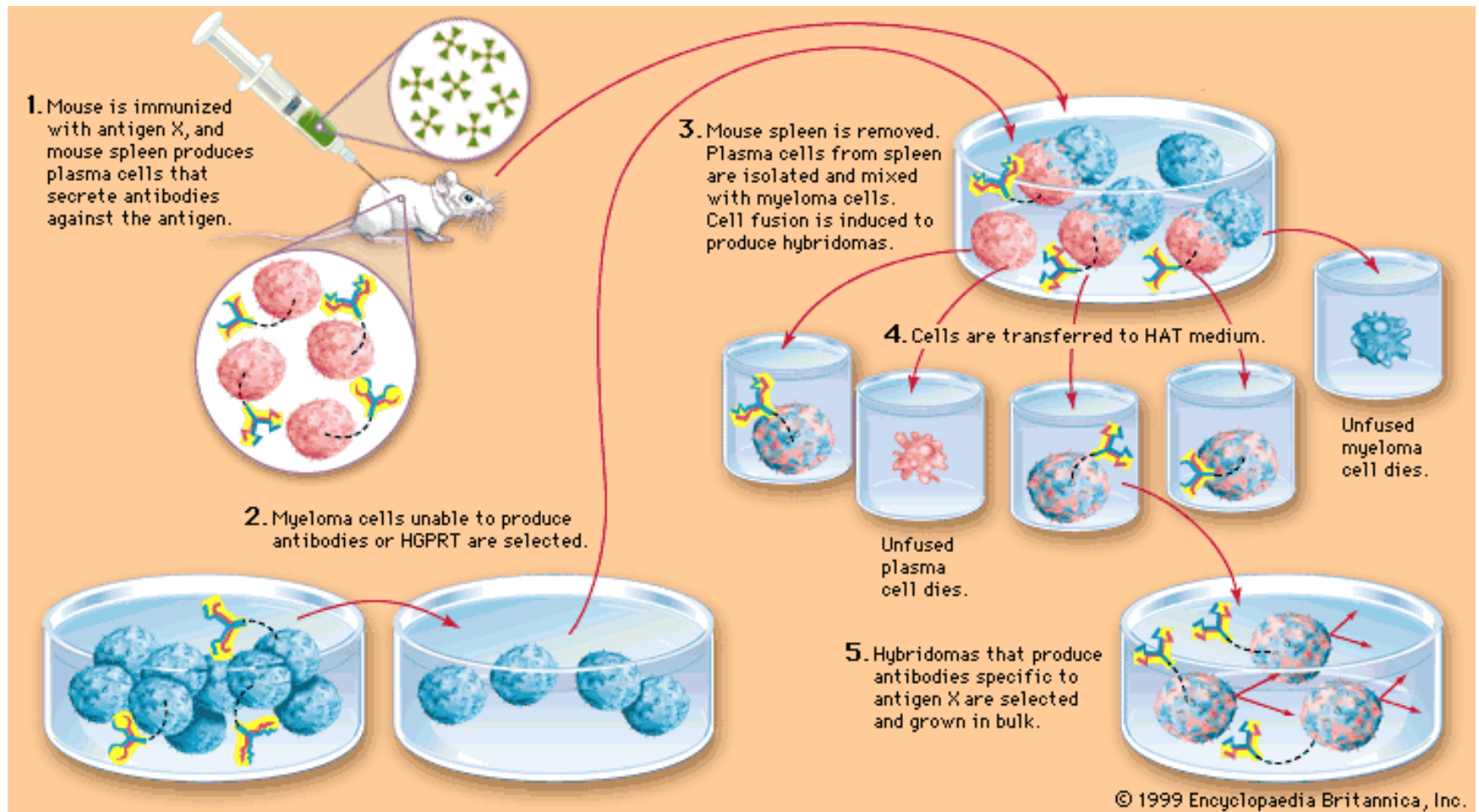




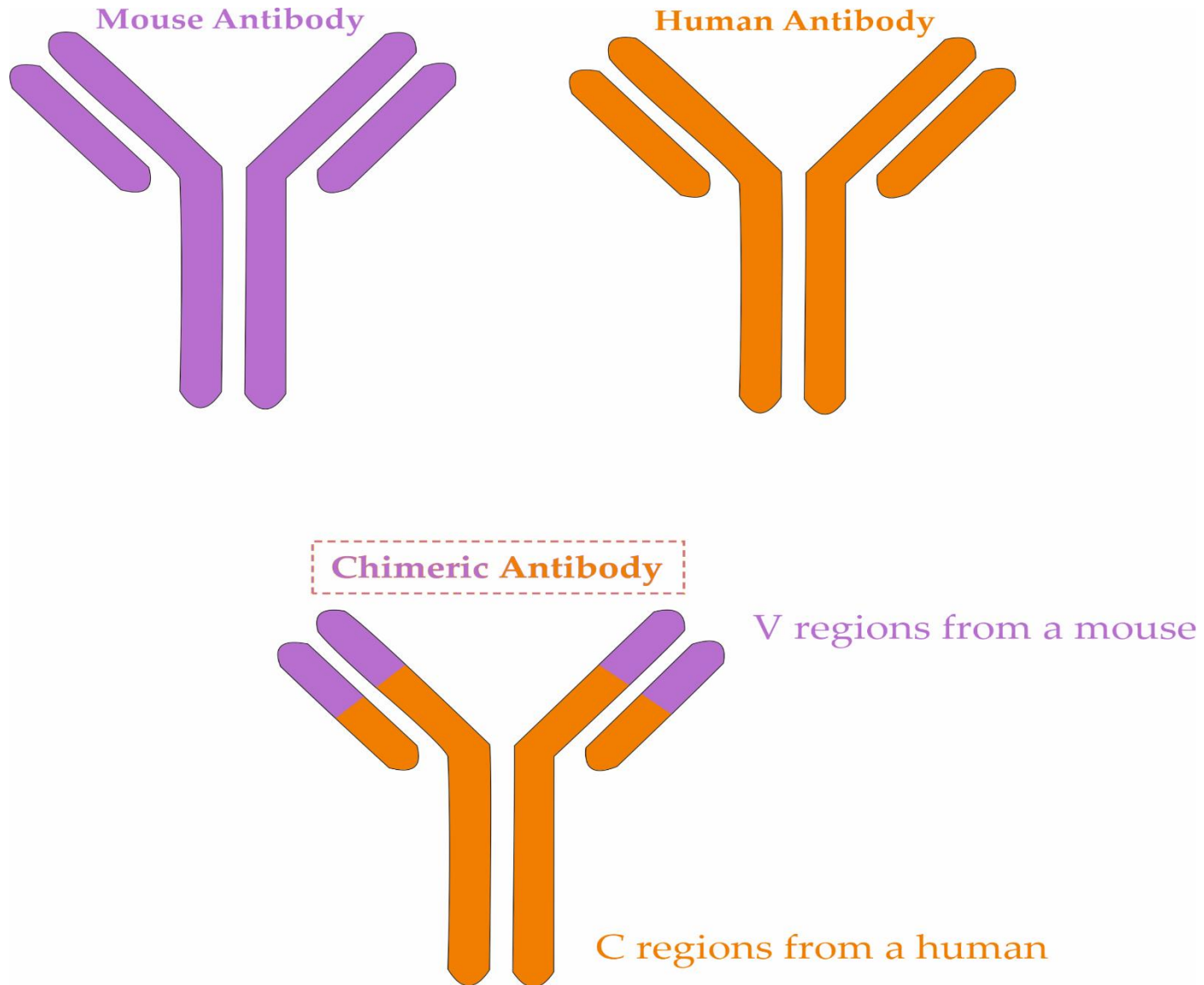
# Hybrid Cell Technique

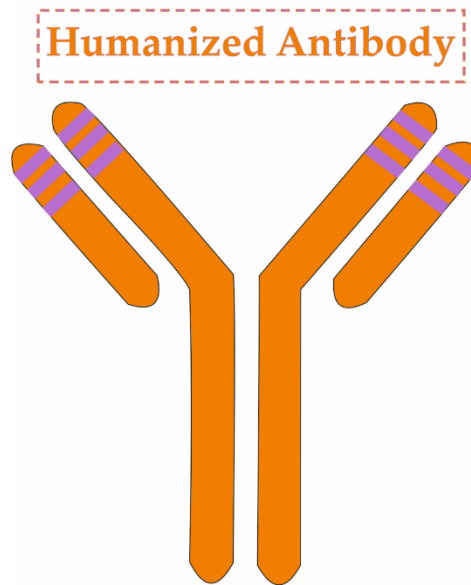
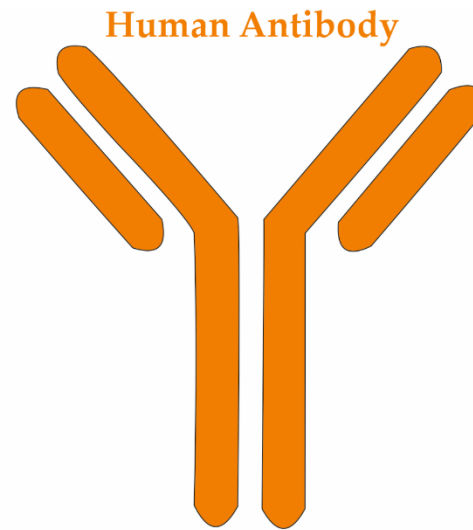
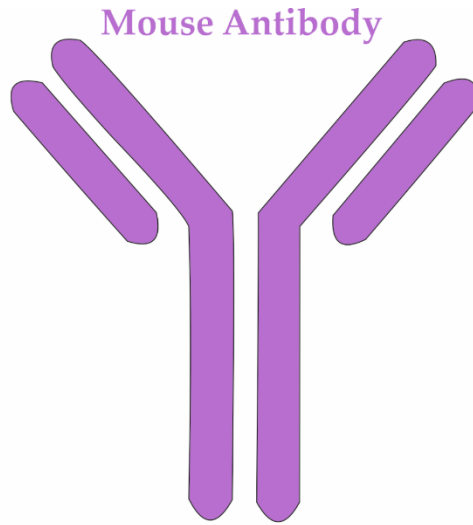


# Mouse Monoclonal Antibodies Production Technology

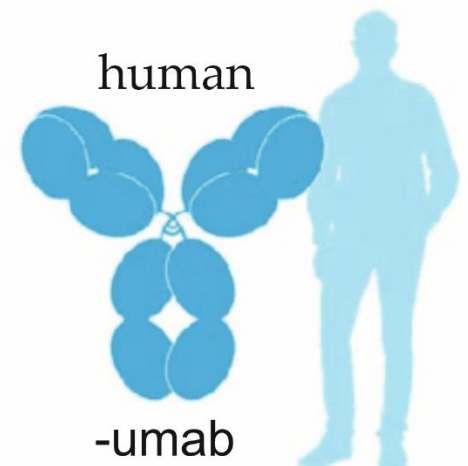
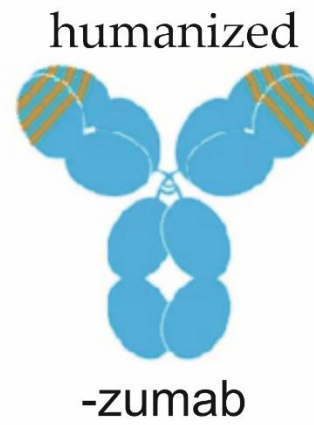
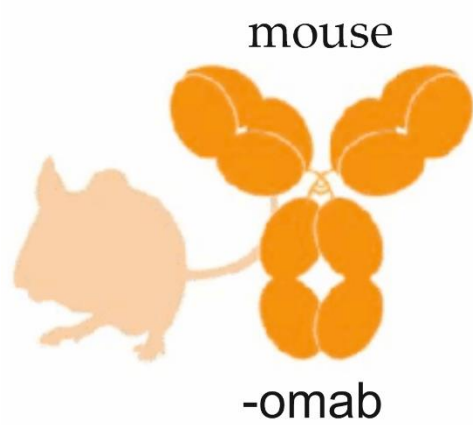


*Genetic engineering techniques for obtaining different forms of humanized antibodies contributed a lot in order to solve all the previous mentioned problems.*





Humanized antibodies are obtained by merging hypervariable parts of the mouse antibody with the rest regions of human antibody. In other words, in variable human antibody regions, mouse hypervariable regions are inserted.



# Antibodies

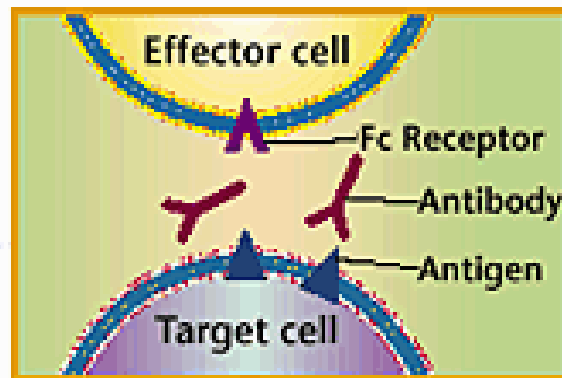
## Monoclonal antibodies

- Specific for oncogenic products that are expressed at high levels in some tumors
- Enhancing the efficacy of mAb (mouse):
- humanized antibodies
- Hybrid antibodies retain their antigenic specificity and have other characteristics of human antibodies
- In order to increase cytotoxicity, radioactive isotopes, cytotoxic drugs or toxins are attached to antibodies.

# Monoclonal antibodies in the treatment of malignant tumors

Monoclonal antibodies can help eliminate malignant cells in several ways.

Antibodies can act by a direct mechanism by binding to receptors important for tumor growth (receptors for IL-2 in leukemias, HER2/neu in cancers...) and thus block the signaling from the receptors that is triggered by the binding of their ligands. Other antibodies bind to and interfere with the function of surface molecules (CD20, CD19 in lymphomas) or activate signaling molecules that cause cell apoptosis (Fas on tumor cells).



**Baseline state**

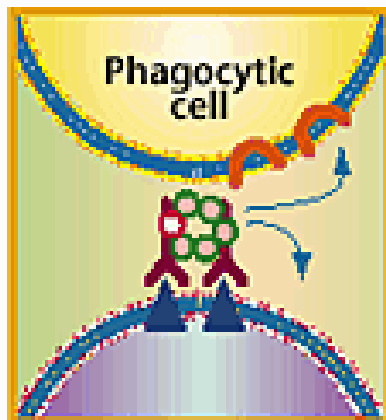
No effector cell engagement  
No complement activation  
No signalling

①

②

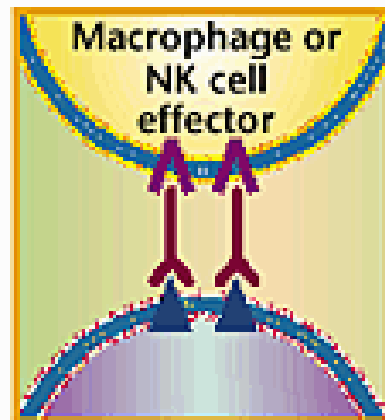
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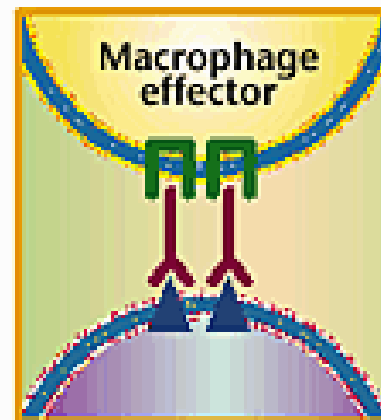
1. Fix complement: initiate opsonization, lysis, inflammation.

**Protection from tumor**



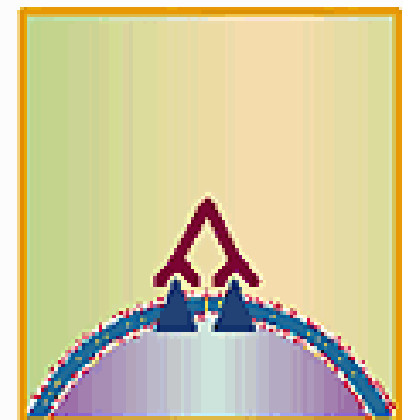
2. Crosslink FcγRIII. Initiate ADCC and cytokine release.

**Protection from tumor**



3. Crosslink FcγRII  
Inhibit effector cell.

**No protection from tumor**

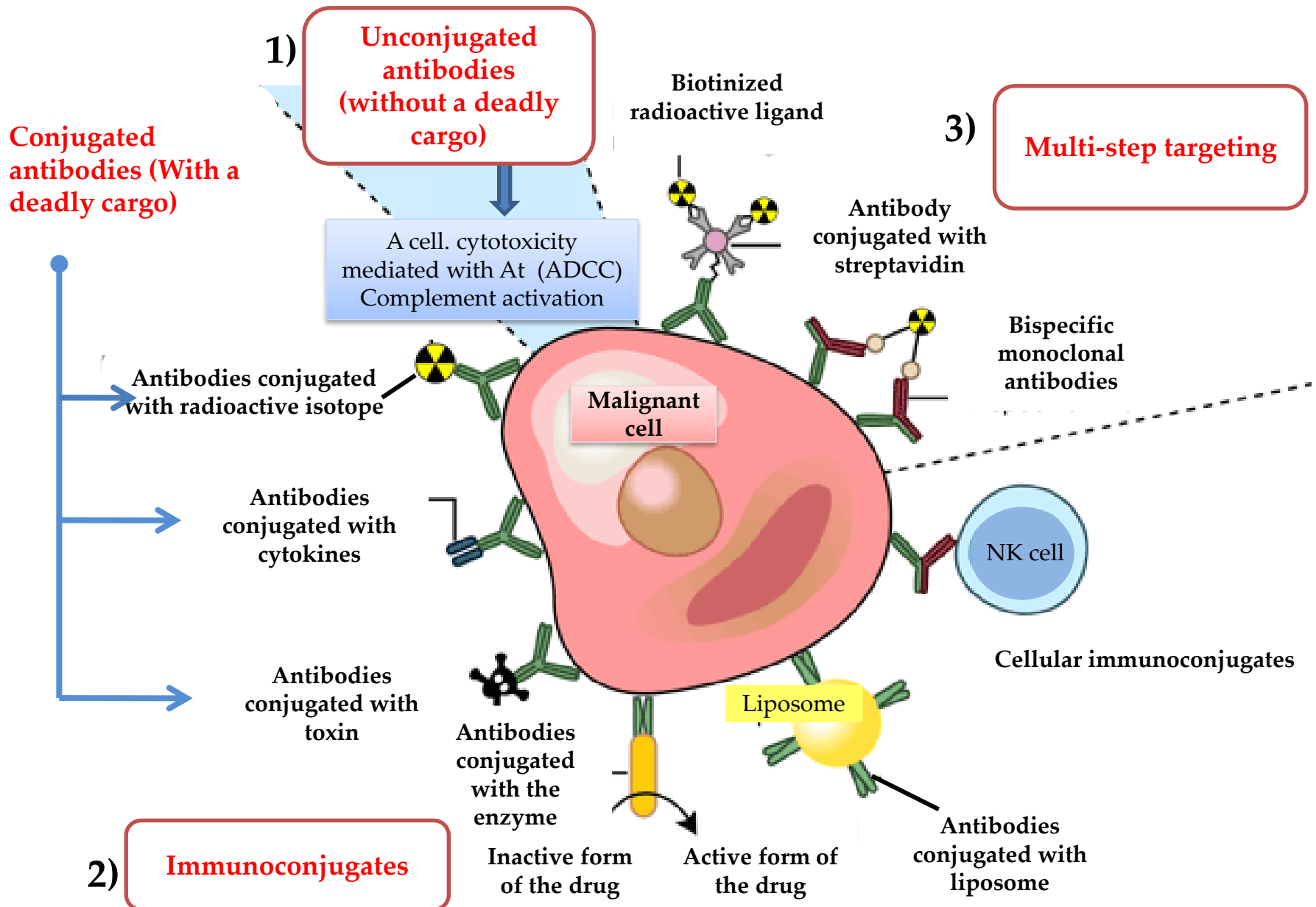


4. Crosslink antigens on cancer cell. Initiate signals, block growth or survival.

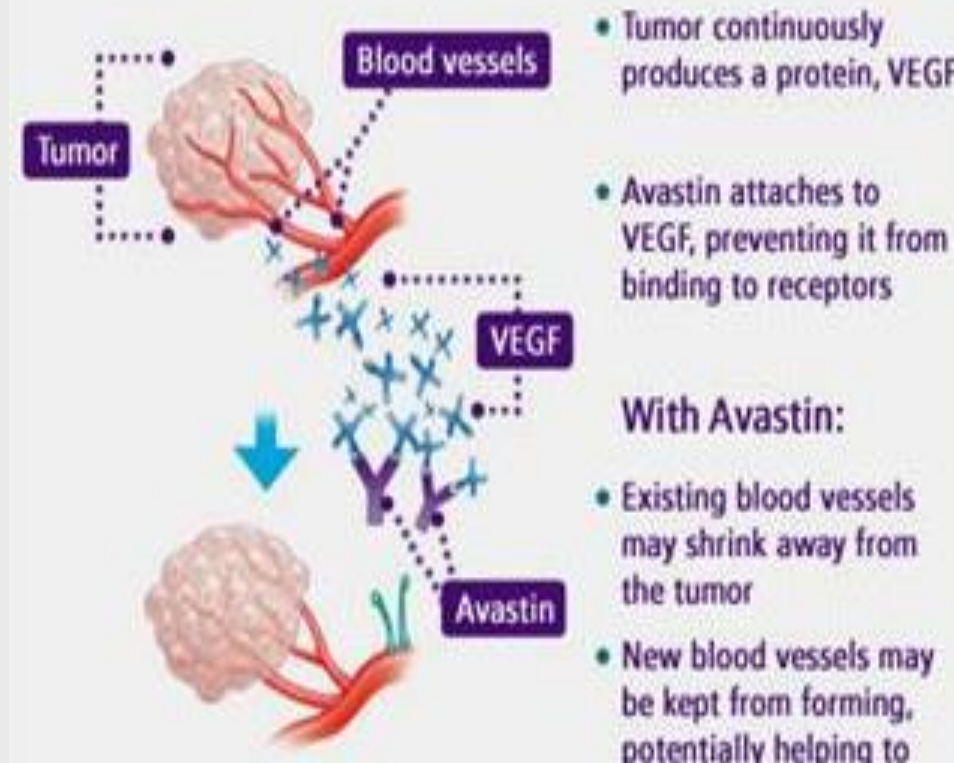
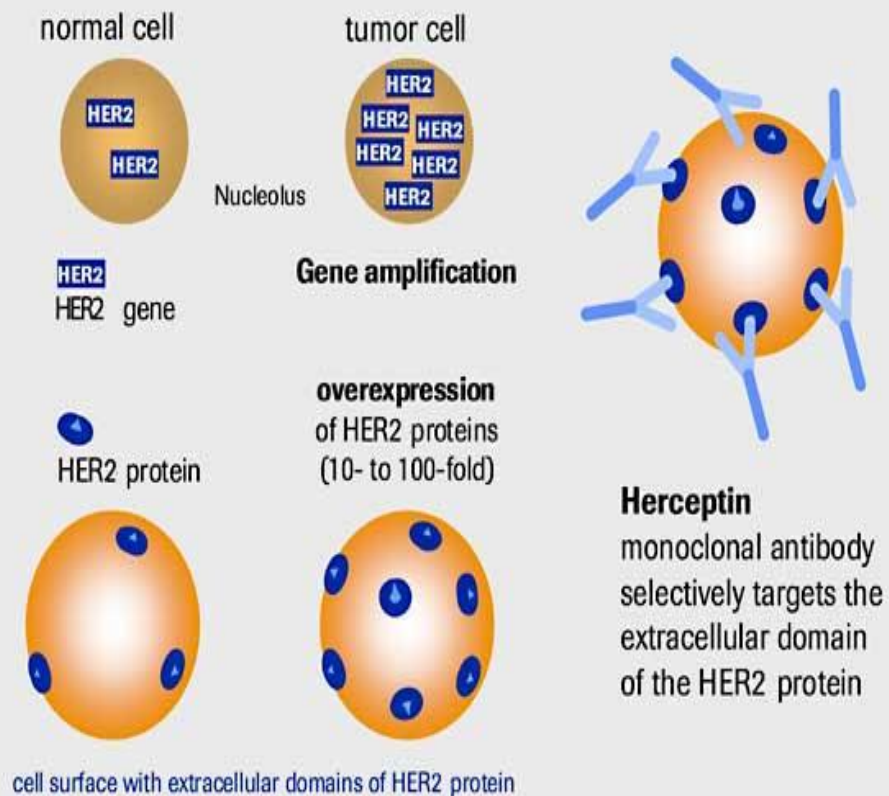
**Protection from tumor**

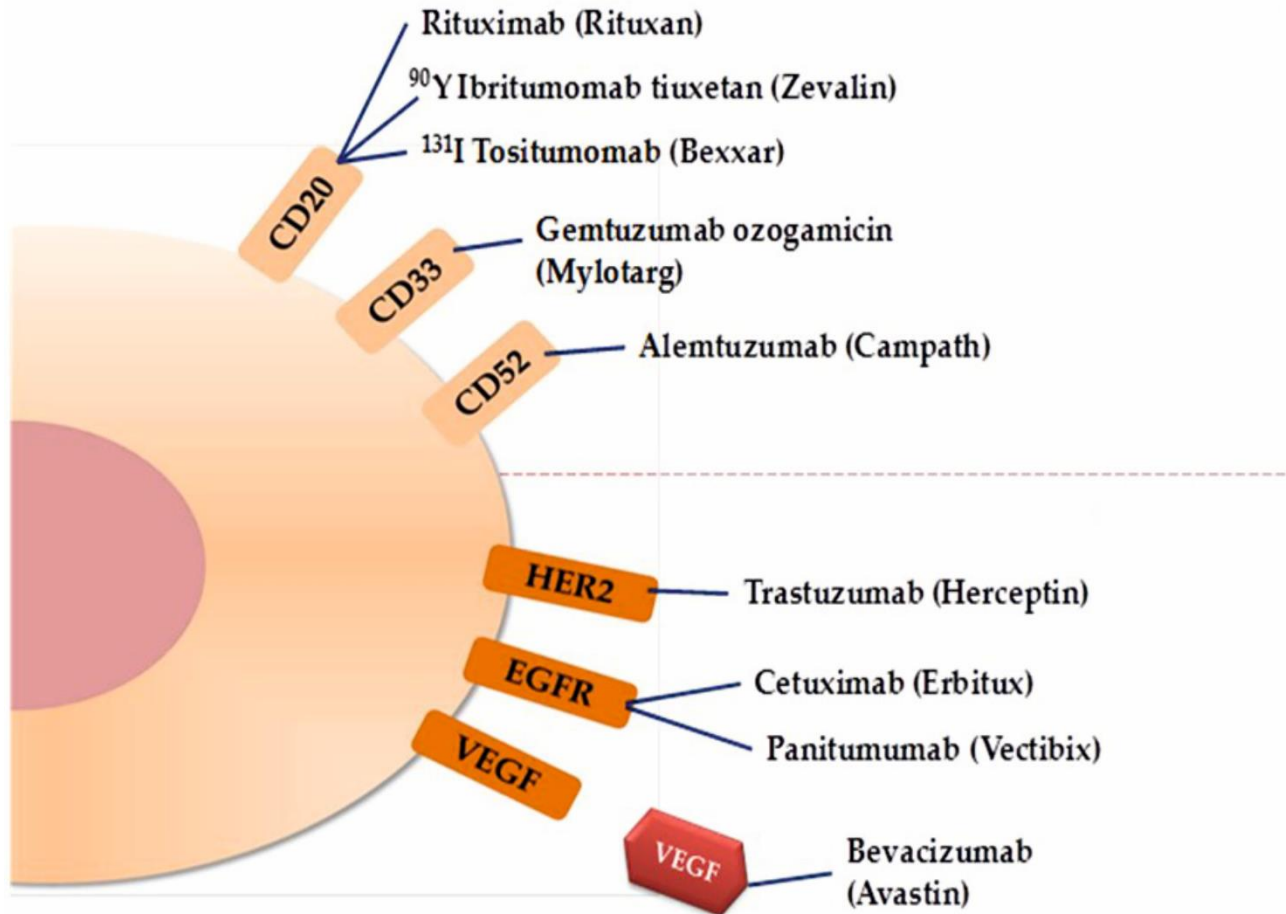


# Antibodies as "Magic Bullets" in the treatment of tumors



## ... Antibodies that inactivate receptors important for tumor growth (HER2) or inhibit growth factors (VEGF) necessary for neoangiogenesis



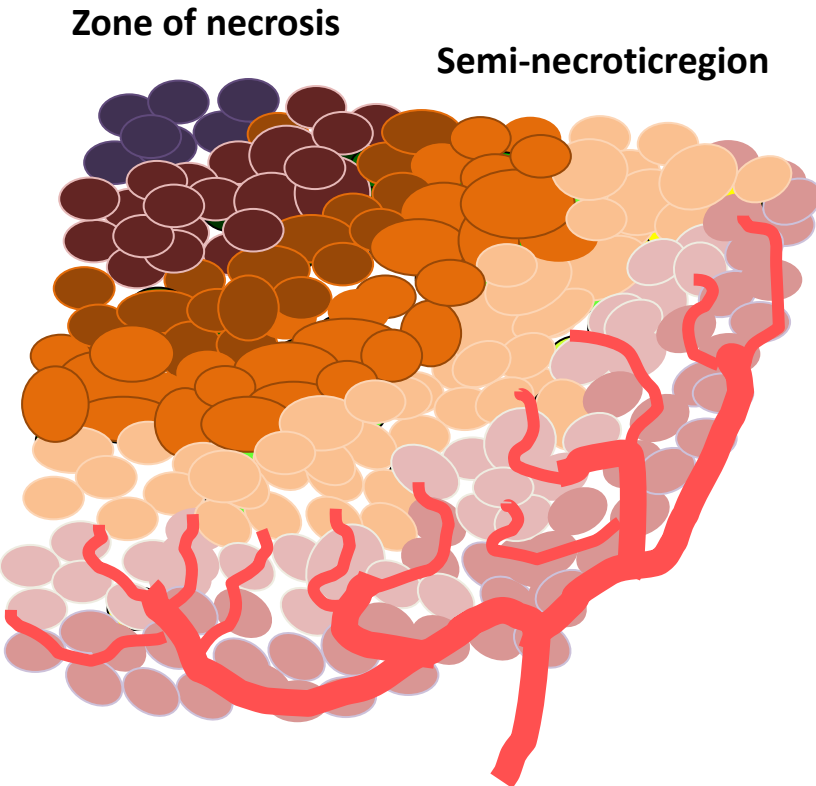


They are in clinical use today and many other monoclonal antibodies:

- Antibodies specific to tumor antigens (Gemtuzumab ozogamicin – CD33),
- Antibodies that inactivate receptors important for tumor growth (Herceptin - HER2)
- Inhibits growth factors necessary for neo-angiogenesis (Avastin - VEGF)

# Problems limiting success in tumor therapy

Main problem that limits the success of immunotherapy is that many tumors do not express specific antigens, so there is a possibility of unwanted, cross-reactivity with normal tissue.



Penetration of monoclonal antibodies into tumor tissue is influenced by the size of the tumor mass, the permeability of the vascular network and the characteristics of the tumor microenvironment such as pH, pO<sub>2</sub> and increased pressure of interstitial fluid.

# LAK cells

- *In vitro* cultivation of mononuclear cells in the presence of high concentrations of cytokines leads to the formation of the so-called lymphokine activated killer cell (LAK)
- NK cells but also cytotoxic T lymphocytes
- Greater killing capacity than the original population
- They return to the affected person with further *in vivo* administration of IL-2
- Immunotherapy gave impressive results in the regression of solid tumors

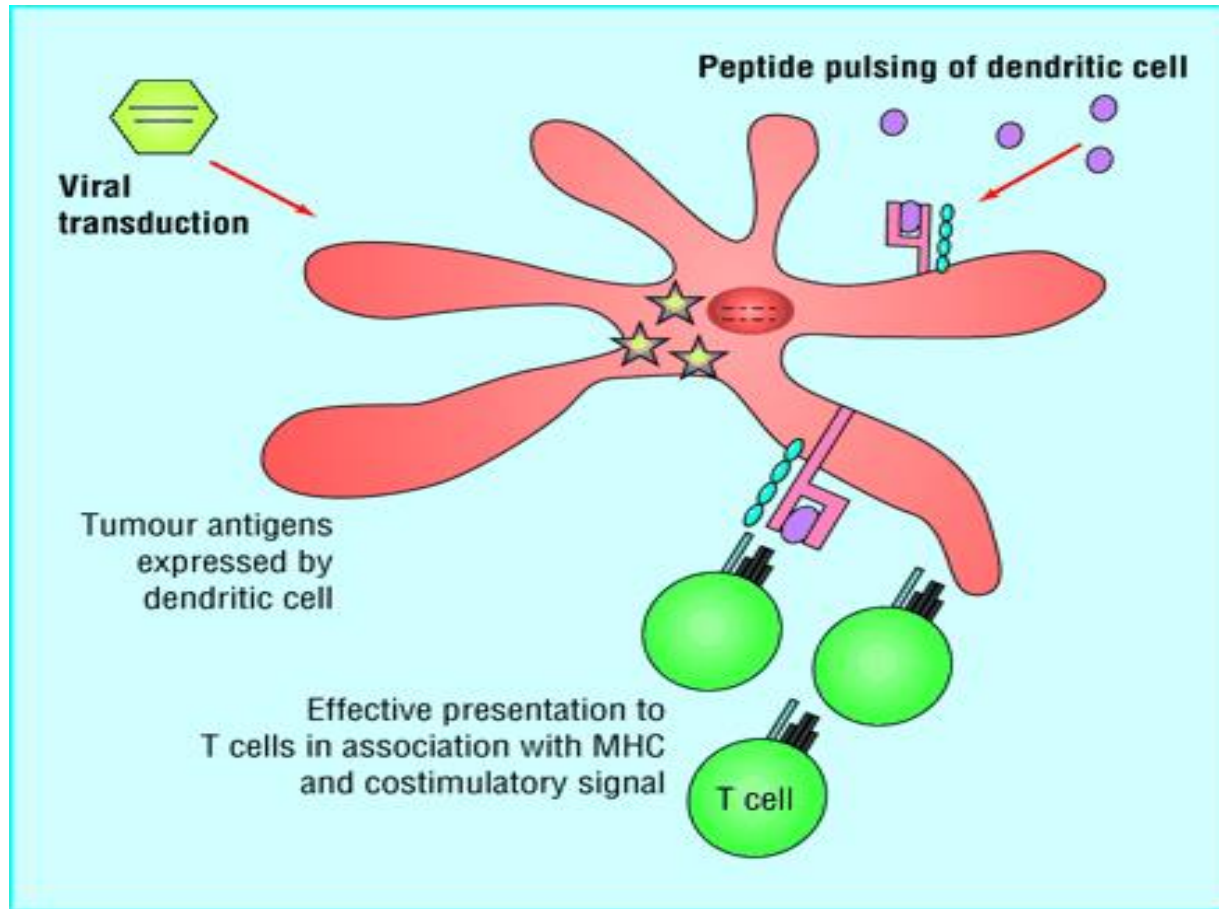
# TIL cells

- TIL therapy
- In 1986, Rosenberg isolated lymphocytes that infiltrate tumors – TIL
- Tumor-specific CTL capable of killing 100–1000 times more efficiently than LAK cells after in vitro activation with IL-2
- TIL: pulverization of the tumor to a single-cell suspension of mononuclear cells and tumor cells which is then cultured in vitro for 30 to 45 days with anti-CD3 mAb and IL-2 until the required number of TIL cells is reached, which are then infused with IL-2 back to the patient.

# Specific active immunotherapy

- Vaccination
- Tumor cells and antigens
- Increasing the immune response against tumors the best way to induce CTL responses
- It is difficult to induce a strong and sufficient immune response that will eradicate all the cells of a growing tumor
- Development of virally induced tumors can be prevented by vaccination with viral antigens or diluted live viruses

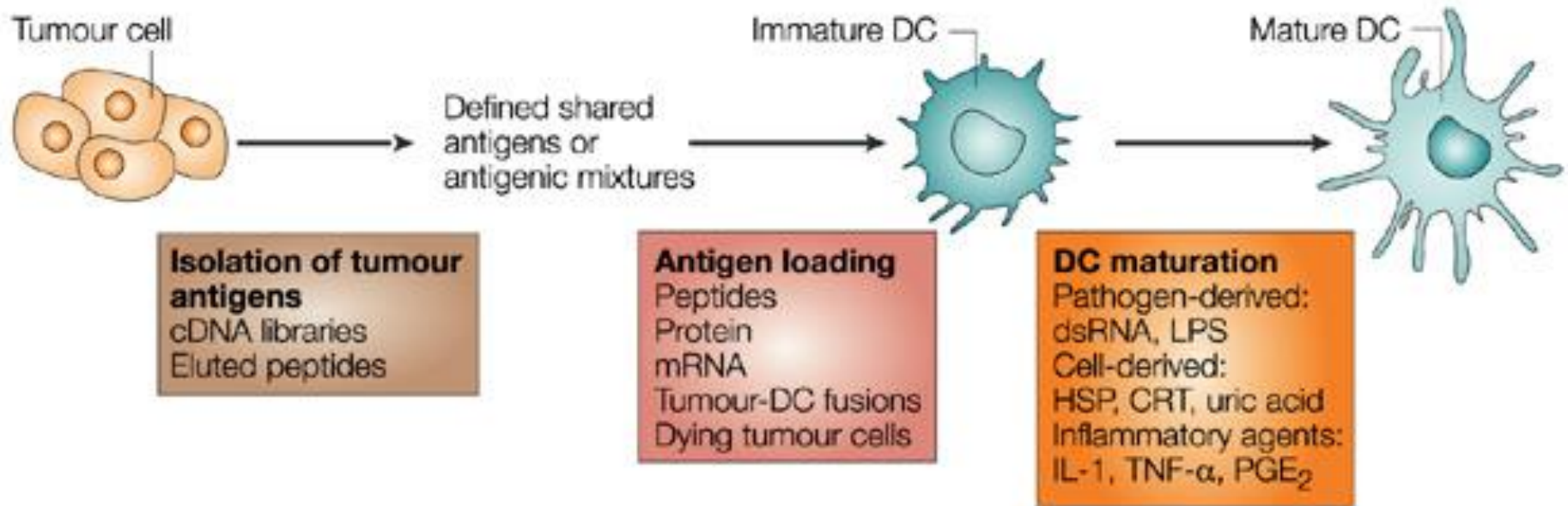




immunization with purified dendritic cells  
incubated with tumor cells



# *Ex vivo generation of dendritic cells*

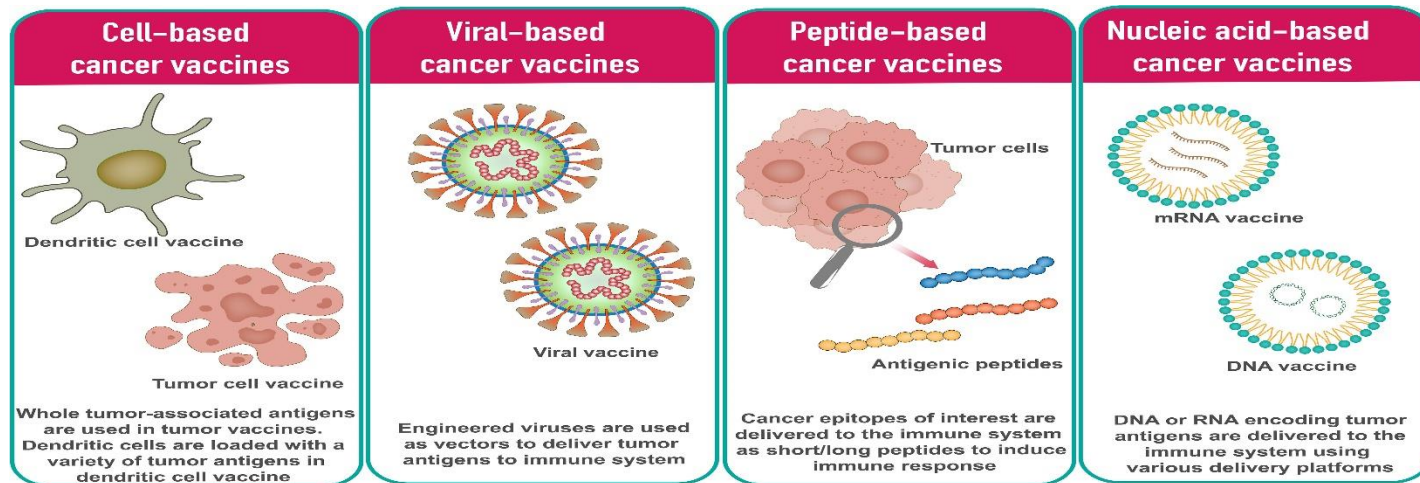


Nature Reviews | Cancer

- Isolated tumor antigens (surface)
- DCs incubated with tumor antigens
- Maturation (calreticulin, HSP, interleukin-1; LPS, PGE<sub>2</sub>, TNF

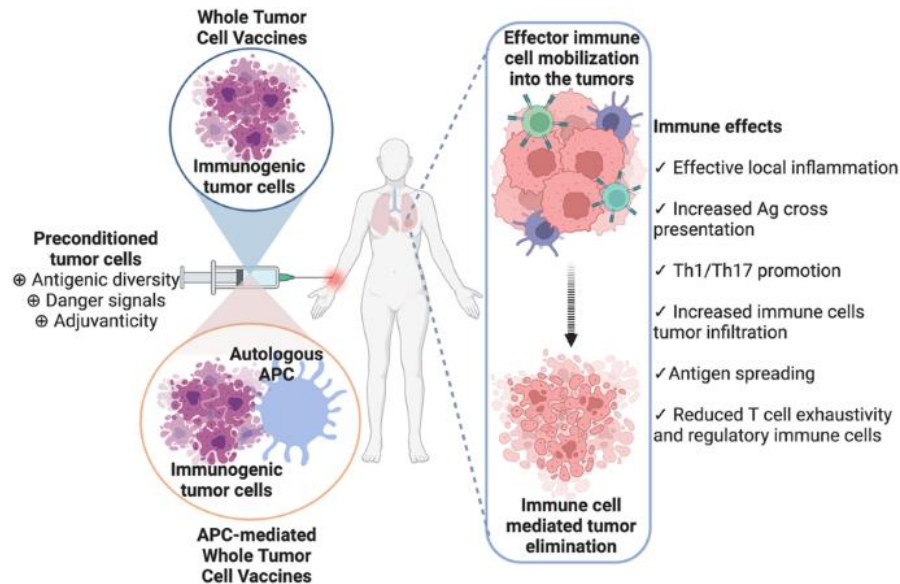
# Four Categories of Cancer Vaccines

- Cell-based
- Peptide-based
- Viral-based
- Nucleic acid-based



# Cell-based Cancer Vaccines

Cell-based cancer vaccines are often prepared from whole cells or cell fragments



## Immune Cell Vaccine

- Immune cell vaccine is based on cells role in the immune system.
- Import tumor-associated antigens into DCs to make them play the role of antigen presentation and activate T cells.
- Exosomes released by DCs (**DCexos**)

## Tumor Cell Vaccine

- Tumor cell vaccine contains the whole tumor-associated antigens (epitopes for CD4+ helper T cells and CTLs)

# Virus-based Cancer Vaccines

- Viruses are naturally immunogenic and their genetic material can be engineered to contain sequences encoding tumor antigens
- Several recombinant viruses, such as adenovirus, can infect immune cells as vectors. The engineered virus vaccines can present tumor antigens in large quantities in the immune system and produce anti-tumor immunity
- Oncolytic virus can be used as a vector as well. Except for providing tumor antigens, the virus itself can also lyse the tumor, release tumor antigens, further increase the vaccine's effectiveness, and produce long-term immune memory

# Virus-based Cancer Vaccines

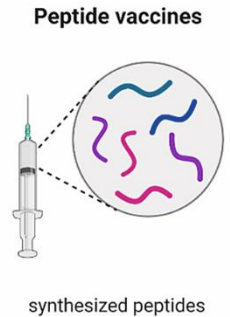
Primary benefits of virus-based vaccines is that the vaccine can make innate and adaptive immune work together to achieve an effective and long-lasting immune response

**Three forms** of the virus based vaccines:

- Inactivated, live attenuated, or subunit vaccines against the virus that can cause the tumor
- Oncolytic virus vaccine
- Virus vector vaccine

# Peptide-based Cancer Vaccines

- Peptide-based subunit vaccines, including chemical and biosynthetic preparations of predicted or known specific tumor antigens, induce a robust immune response against the particular tumor antigen site.
- Peptide-based subunit vaccine combined with adjuvants can efficiently provoke humoral immune response, suitable for preventing and treating viral infectious diseases. HBV and HPV vaccines for liver and cervical cancers were primarily peptide-based subunit vaccines



# Nucleic acid-based Cancer Vaccines

- Nucleic acid vaccine induces strong **MHC I** mediated **CD8 + T cell** responses; Desirable cancer vaccine platform
- Nucleic acid vaccines can simultaneously deliver multiple antigens to trigger humoral and cellular immunity
- Nucleic acid vaccines can encode full-length tumor antigens, allowing APC to cross-present various epitopes or present several antigens simultaneously
- Nucleic acid vaccine preparation is simple and fast, which is suitable for developing personalized neoantigen cancer vaccines

- The basis of all immunotherapy and cancer vaccines is a comprehensive understanding of the tumour's immune evasion mechanisms.
- Emerging technology has made it possible to dissect the TME in depth and draw significant conclusions regarding the mechanisms of intrinsic and extrinsic resistance governing the response to therapy at various stages of disease.