(Selected slides)

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Monoclonal antibodies and cancer immunotherapy

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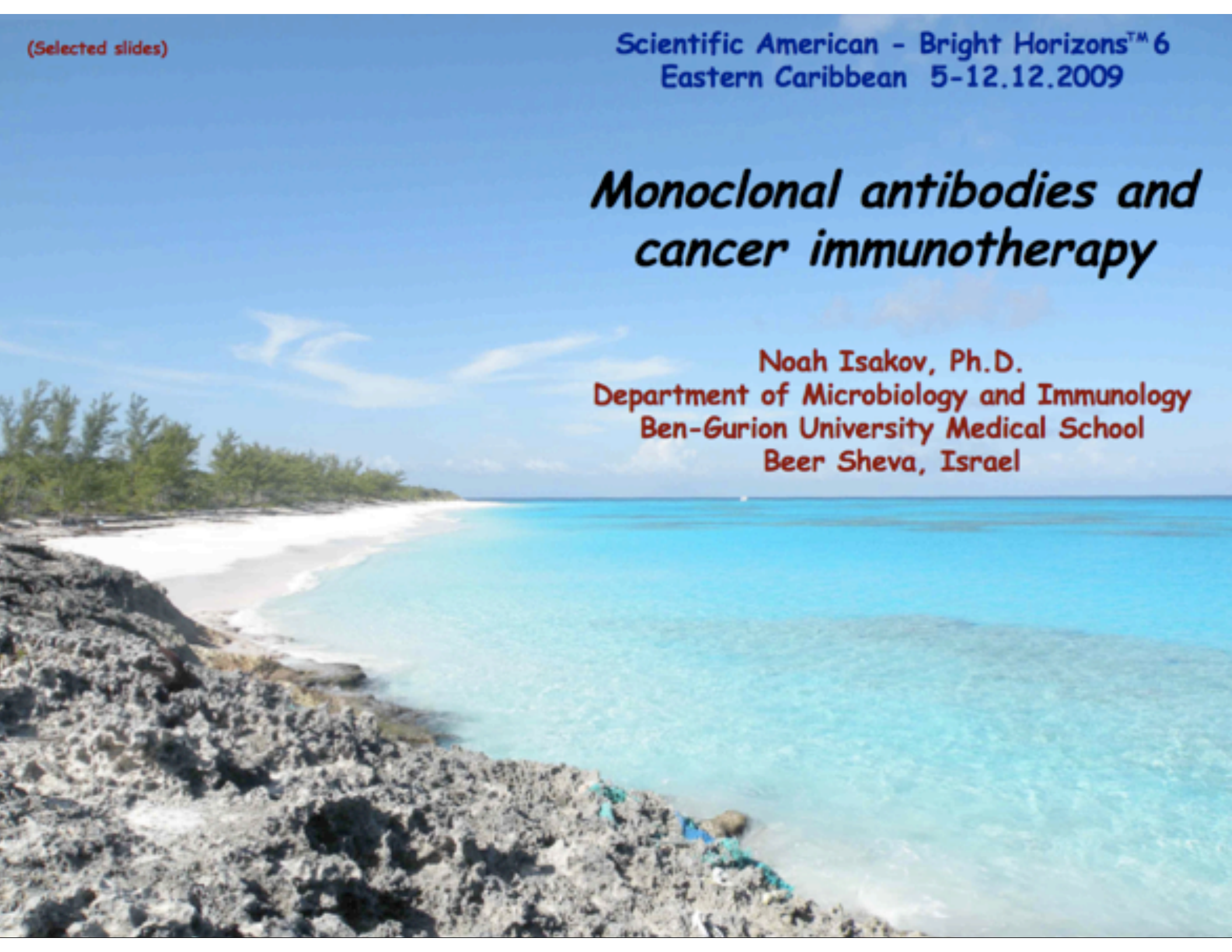


Major milestones in the history of immunology

430 B.C. - Thucydides observed that people who recovered from plague could nurse the sick because they were protected from re-infection.

1798 - *Active immunization*: Dr. Edward Jenner inoculated a child with pus from a cowpox, challenged him with smallpox and observed full immunity. First example of active immunization.

1880 - Louis Pasteur showed that injection of live attenuated bacteria induces immunity (Chicken cholera, anthrax, rabies).

1890 - *Passive immunizaiton:* Emil von Behring and Shibasaburo Kitasato independently, showed that immunity to diphtheria and tetanus could be obtained by serum (antibodies) transfer from immune host. First example of passive immunization.

Louis Pasteur observed that injection of an attenuated cholera bacteria protected the host from the disease.

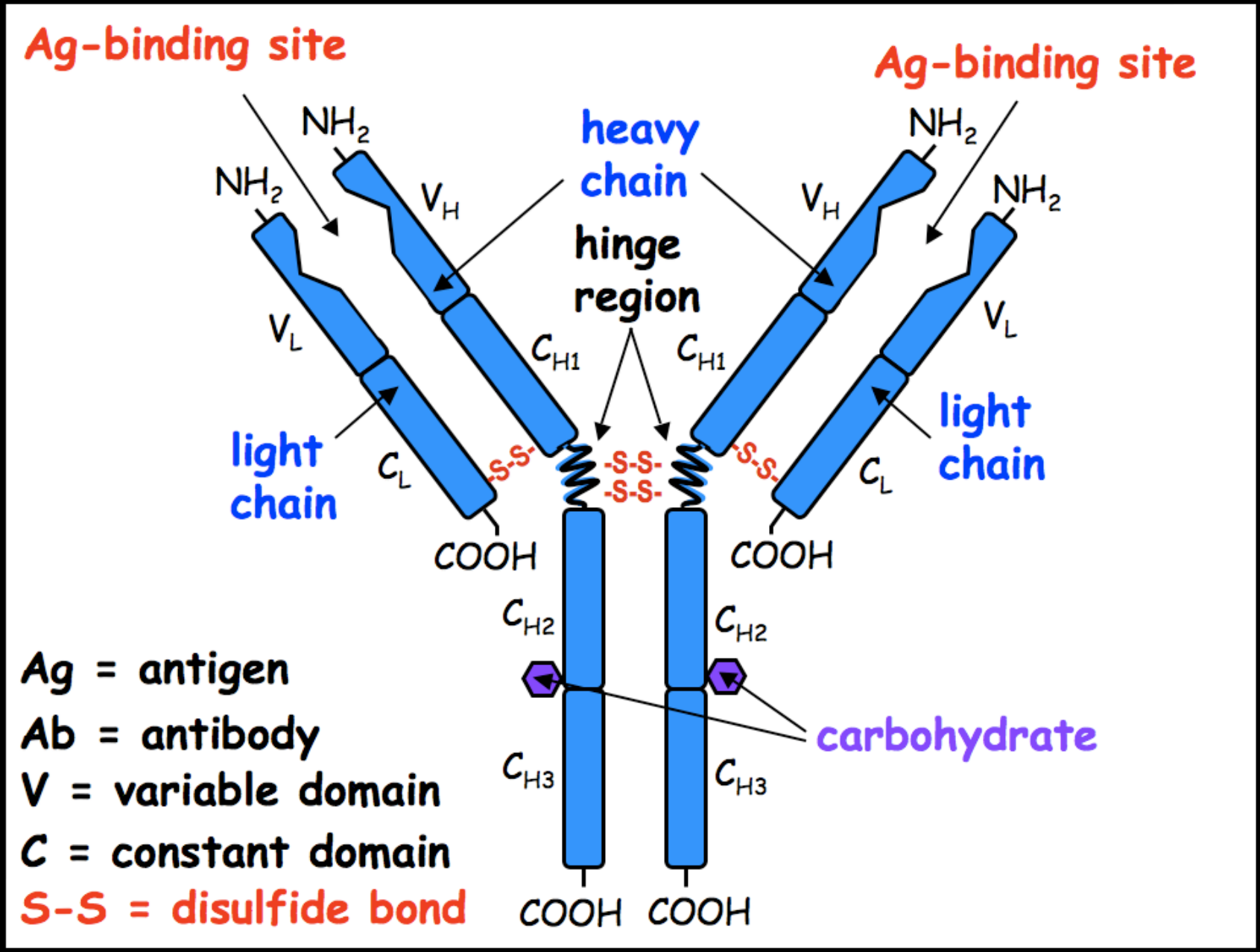
In honour of Jenner's work with cowpox inoculation, Pasteur called the attenuated strain of pathogen 'a vaccine'- from the Latin word 'vacca', and the process of inducing acquired immunity was termed 'vaccination'.

Cowpox = Vaccinia virus.

In 1978 the WHO completed the programme to eradicate Smallpox worldwide.

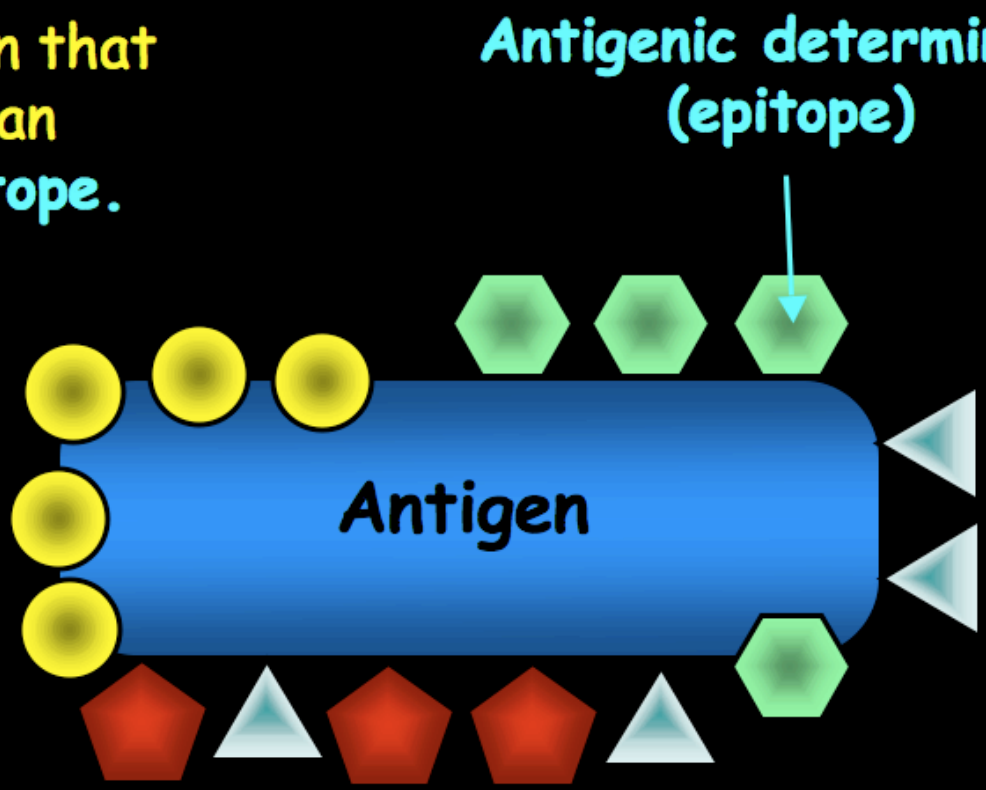
Two main reasons lead to complete eradication of the smallpox: 1. Active immunization of large populations of human beings worldwide. 2. The fact that humans are the only host for smallpox.

IgG: Basic structure of a prototypic antibody molecule



Antigen and antigenic determinants (epitopes)

The specific site of an antigen that binds to an antibody is called an **antigenic determinant** or **epitope**.



A normal antibody response is polyclonal, because most antigens have a variety of epitopes that induce the generation of a number of different antibodies. Such a response is termed polyclonal because it reflects the activation of several different clones of B lymphocytes.

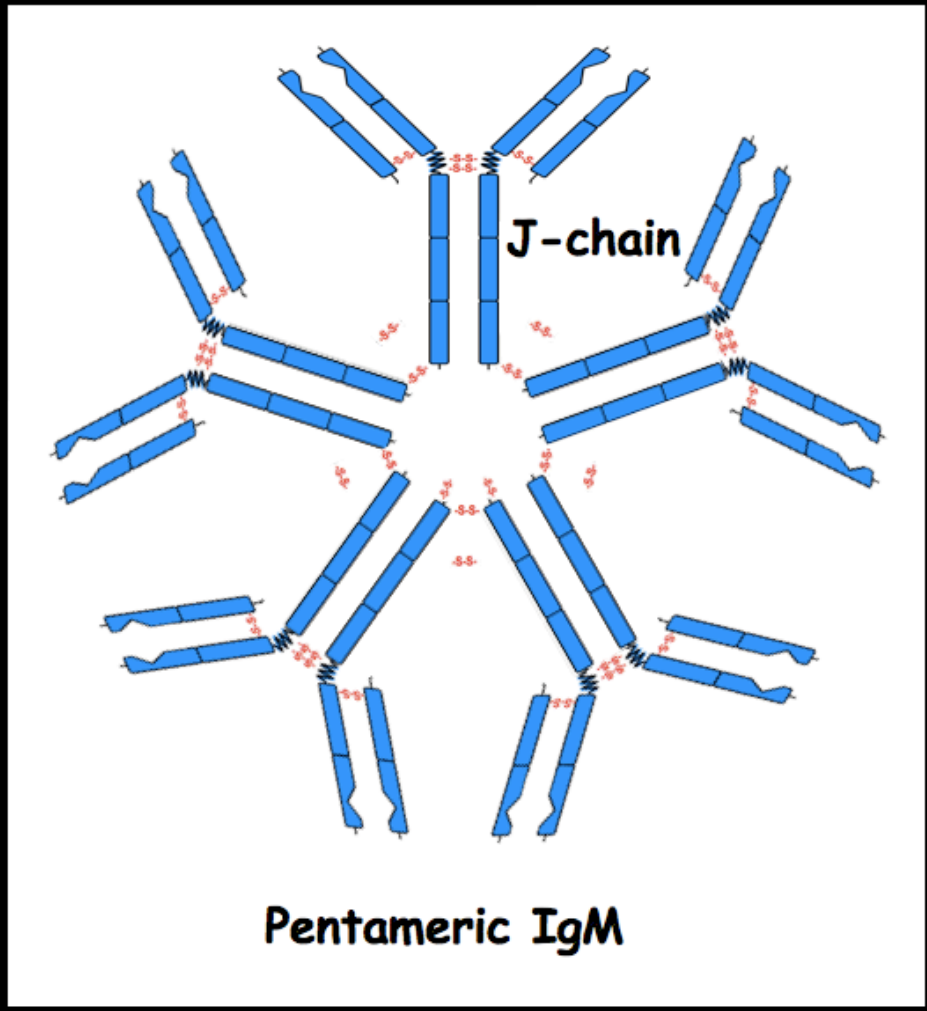
A soluble IgM is a polymer that consists of five IgG-like subunits, or monomers

IgM molecules are synthesized as monomers that undergo assembly to form pentameric molecules. Each pentamer associates with a single 'joining' chain, or J chain.

In a pentameric IgM, the monomers are cross-linked by disulfide bonds to each other and to the J chain.

The Figure shows an IgM pentamer, in which the monomers are arranged as a flat disc. However, the IgM pentamers are flexible and upon interaction with the surface of a pathogen, the IgM can simultaneously interact with up to 10 identical epitopes, acquiring a 'spider'-like shape.

Because of the large size of this antibody, it is termed a Mega immunoglobulin, or IgM.



Soluble IgA antibodies are either monomers or dimers

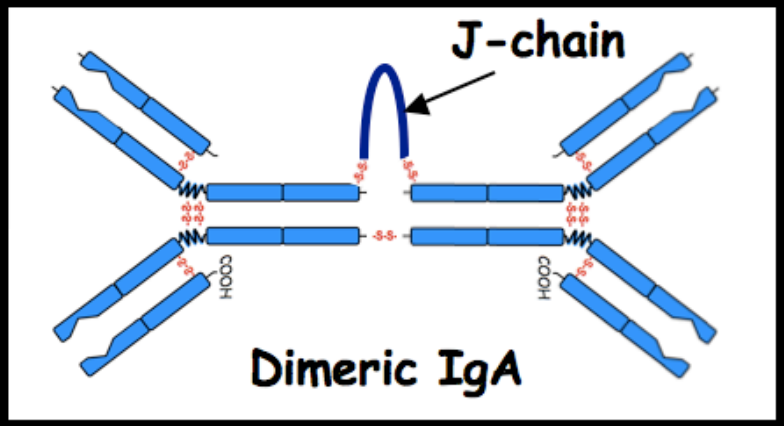
Monomeric IgA antibodies are found in extracellular fluids within the body.

IgA are also synthesized as dimers in association with an additional polypeptide chain, the J chain.

In dimeric IgA, the monomers and the J chain are cross-linked by disulfide bonds.

Majority of dimeric IgA are secreted into tears, saliva, and colostrum, and secretions of the genito-urinary and gastrointestinal tracts, prostate, and respiratory epithelium.

Dimeric IgA play a critical role in mucosal immunity.



Immunobiology, Janeway et al., 4th Ed. Fig. 3.22, p. 104. (5th, Fig. 4.23)

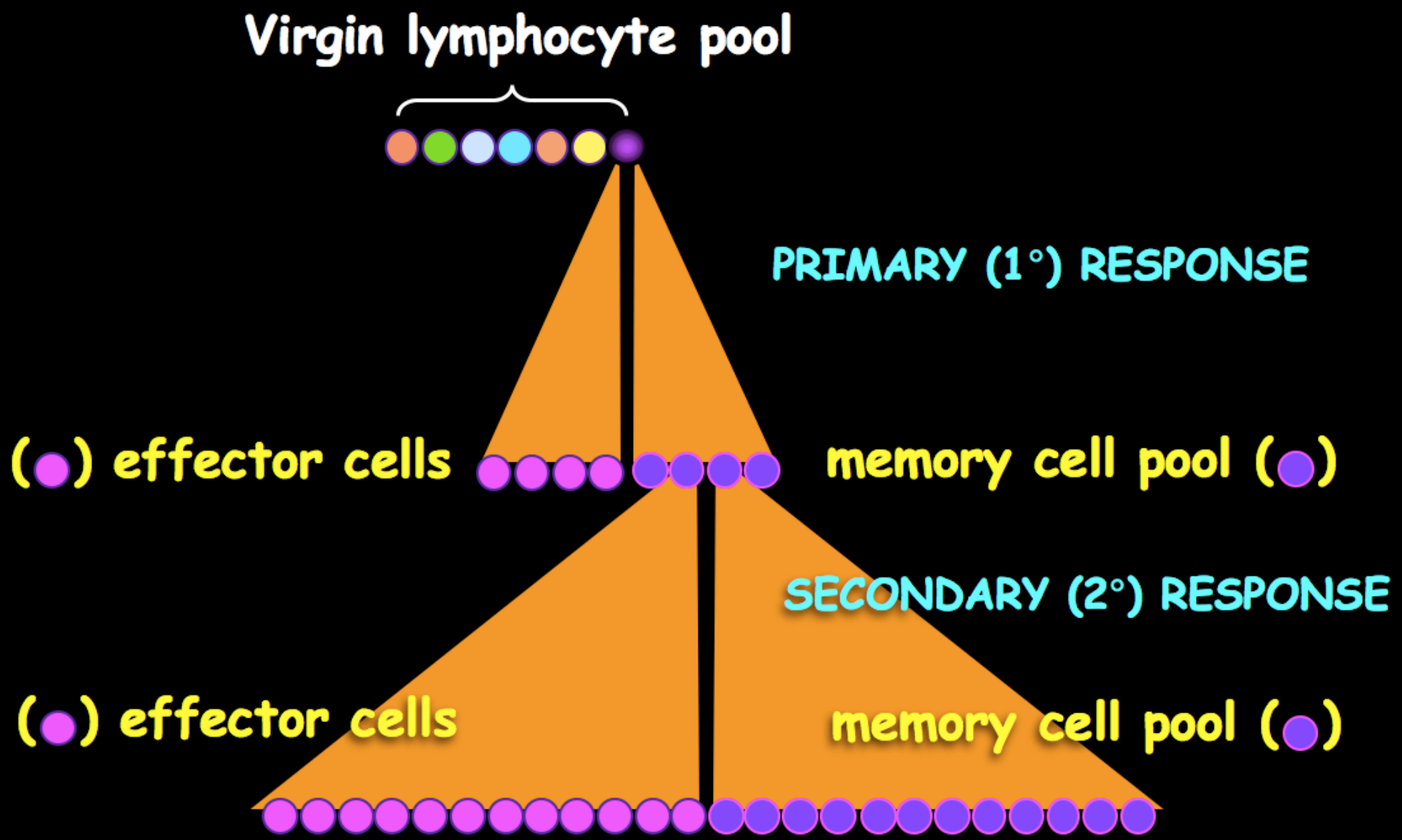
Clonal Selection Theory

The Clonal Selection Theory is the currently accepted model explaining how the immune system responds to infection and how certain types of B and T lymphocytes are selected for destruction of specific antigens invading the body.

The four major postulates of Clonal Selection Hypothesis, are:

1. Each lymphocyte bears a single type of antigen receptor with a unique specificity.
2. Lymphocyte activation is dependent on antigen binding to an appropriate antigen receptor.
3. The differentiated 'daughter' effector cells derived from an activated lymphocyte will bear antigen receptors of identical specificity as the parental cell.
4. Lymphocytes bearing receptors for self molecules will undergo 'negative selection' and be eliminated at an early stage.

Clonal Expansion Following Antigen Exposure



Antibodies

Polyclonal

Abs that are collected from sera of exposed or immunized animal

recognize
multiple antigenic sites
of injected biochemical.

Monoclonal

Individual B lymphocyte hybridoma is cloned and cultured. Secreted Abs are collected from culture media

recognize
ONE antigenic site
of injected biochemical

Generation of monoclonal antibodies (mAbs)

Summary

1. Hyperimmunize a mouse with a specific antigen.
2. Fuse spleen cells from the hyperimmunized mouse with cells of an Ig-non-secreting (HGPRT-deficient) myeloma B cell line, using polyethylene glycol (PEG) as a cell fusion reagent.
3. Culture of fused cells under limiting dilutions (in 96 well plates) in the presence of a HAT selection medium.
4. Screening of suitable cell lines.

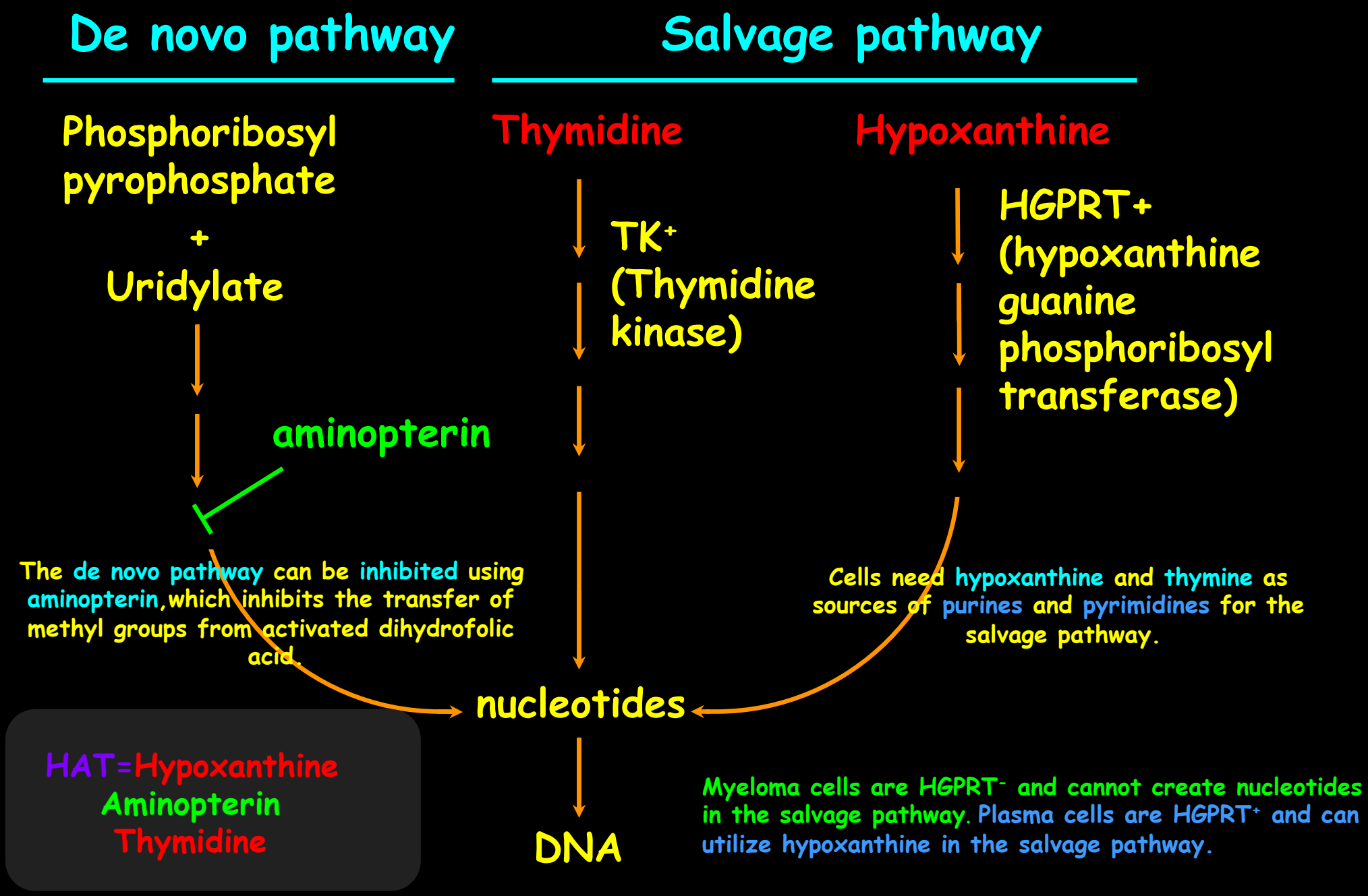
HAT medium (hypoxanthine, aminopterin, thymine).

In culture, individual B cells or fused normal B cells will die, because they are mortal, and can not proliferate in vitro for more than few days.

In the presence of HAT culture medium, the immortal tumor cells or fused tumor cells will die, because they are HGPRT-deficient and cannot utilise the salvage pathway for nucleotide synthesis.

Only fusions of normal B cells and tumor cells will stay alive and propagate in vitro because they are HAT resistant and immortal.

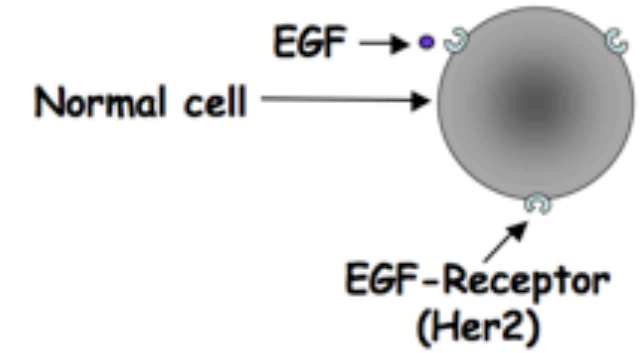
Metabolic pathways leading to nucleotide synthesis



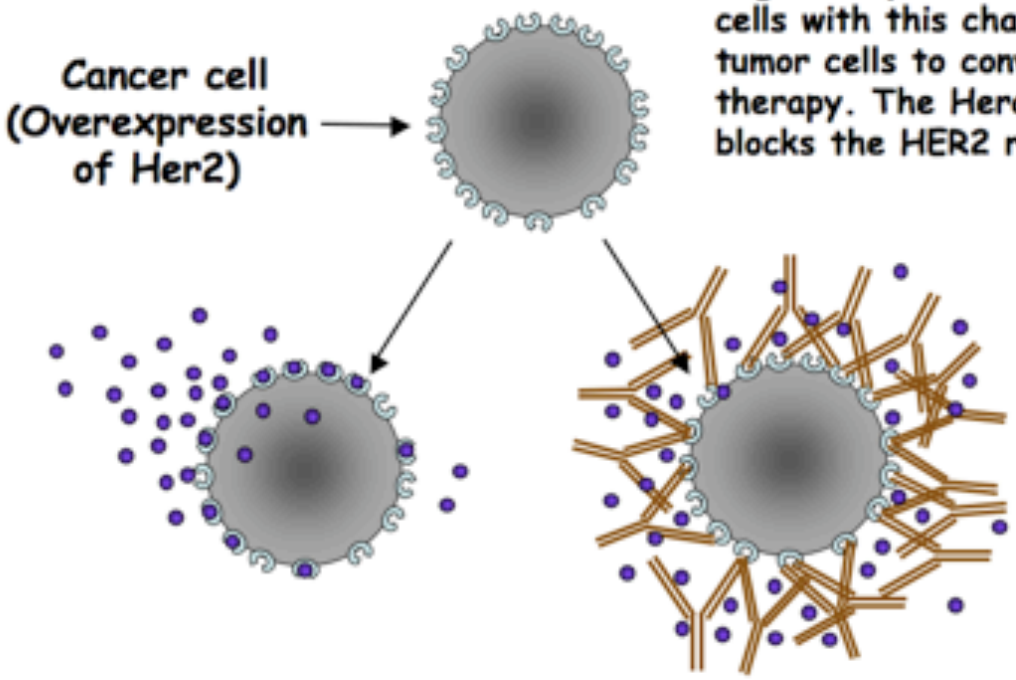
Advantages of monoclonal Abs

- ✓ Time and money saving when large quantities are required.
- ✓ Needs only small amounts of pure Ag for the initial immunization and screening.
- ✓ Standardization: Can make an infinite number of identical tests to be used worldwide.
- ✓ An infinite and unlimited source: mAb-producing hybridoma cells can be stored at -170°C indefinitely. Cells can be grown on industrial scale to produce very large quantities of mAbs.
- ✓ Can be manipulated, modified, and improved by methods of genetic engineering.
- ✓ mAbs are specific for a single epitope and therefore can be used for discrimination between virus subtypes or other crossreactive antigens.
- ✓ Can be selected according to required properties, such as neutralizing mAbs, cytotoxic mAbs, etc. In contrast, polyclonal Abs include a mixture of Abs with different biological activities.

Anti-tumor immunotherapy using tumor antigen-specific monoclonal antibodies



Each year around half a million women throughout the world develop breast cancer, a disease that accounts for almost 20 percent of all deaths in women.
Among patients with metastatic breast cancer, 25-30% express very high levels of HER2 (human epidermal growth factor receptor 2) protein on the tumor cells. This is generally due to amplification of the HER2 gene. Tumor cells with this characteristic respond less well than other tumor cells to conventional chemotherapy and hormonal therapy. The Herceptin monoclonal antibody specifically blocks the HER2 receptor and turn off the EGF-induced "on" signal for cell division, permanently. Herceptin is effective in increasing the survival time of women with advanced breast cancer.



Herceptin, a Her2-specific monoclonal antibody

EGF-induced rapid cell growth

Anti-Her2 blocks EGF-receptors making the cell refractory to EGF

Antibody-dependent immunotherapy is being used for preventive and therapy medicine

- Passive immunization (i.e., against snake venom).
- Infusion of anti-Rh Abs to pregnant, Rh^- women, bearing Rh^+ embryos, to prevent the formation of hemolytic disease of the newborn.
- Utilization of Abs for negative selection of T cells from a transplantable bone marrow.
- Infusion of anti-cancer cell Abs.

  Infusion of anti-cancer cell Abs bound to toxins, isotopes, or drugs.

  Infusion of Abs against viral antigens (i.e., HIV), to neutralize viruses

  Infusion of Abs against cellular receptors for viruses (i.e., HIV), block the receptor and prevent further infection.

  Infusion of Abs against TNF or other cytokines (or their corresponding receptors), to prevent autoimmune symptoms (i.e., RA)

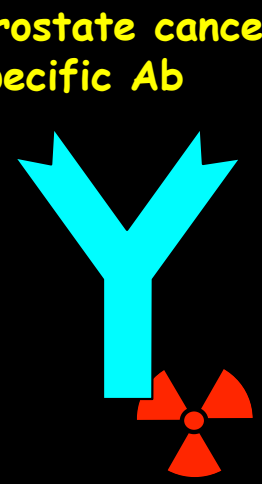
Monoclonal antibodies are being used in cancer diagnostic

Monoclonal antibodies in imaging, therapy assessment, and therapy of solid cancer

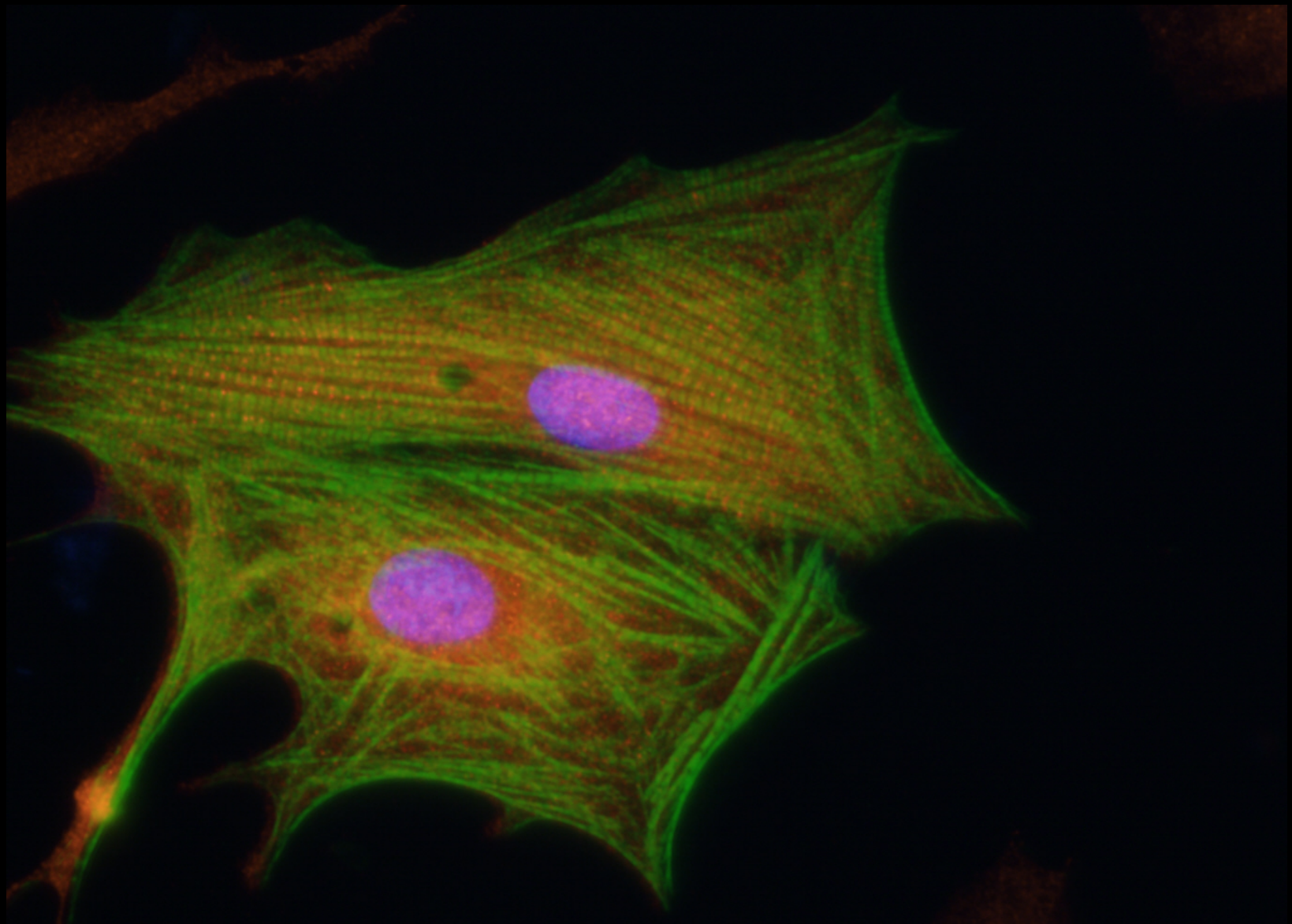
Radiolabeled monoclonal antibodies can be used for imaging of a number of different solid tumors. Radioisotope-labeled monoclonal antibodies specific for a cancer cell antigen, (e.g., prostate carcinoma cells) are being injected into the body of cancer patients, where the antibodies localize at sites of the primary tumor and metastasis. A gamma ray detector is being used for whole body mapping of the tumors.

Such methods are very useful in surgical decision-making regarding tumor resectability. These methods help localizing the primary tomor and additional tumors not readily identified by palpation or inspection, and enable determining surgical resection margins.

New monoclonal antibodies with radiolabels offer hope for more effective agents for imaging, radio-immuno-guided surgery and potential therapeutic modalities.

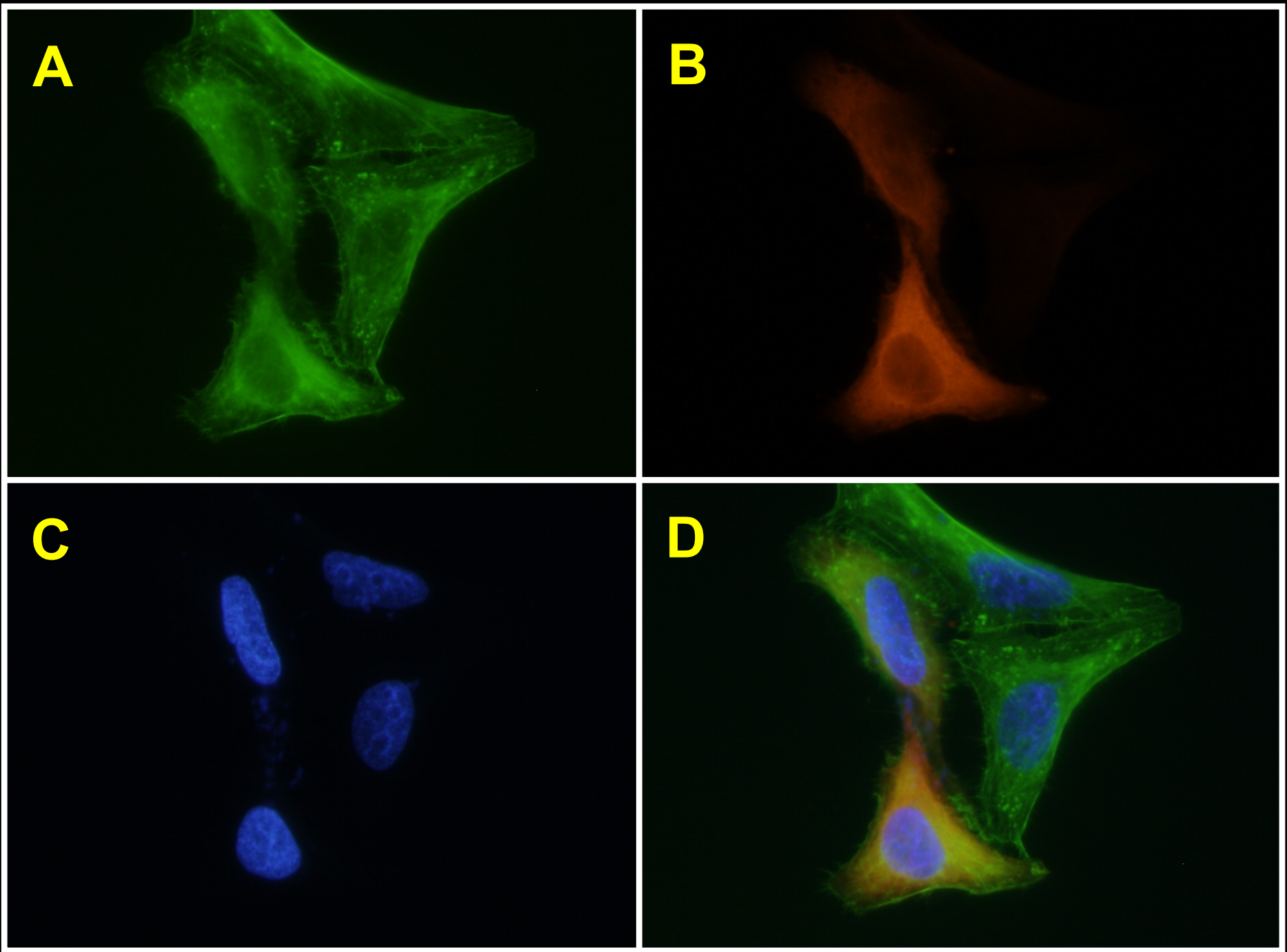


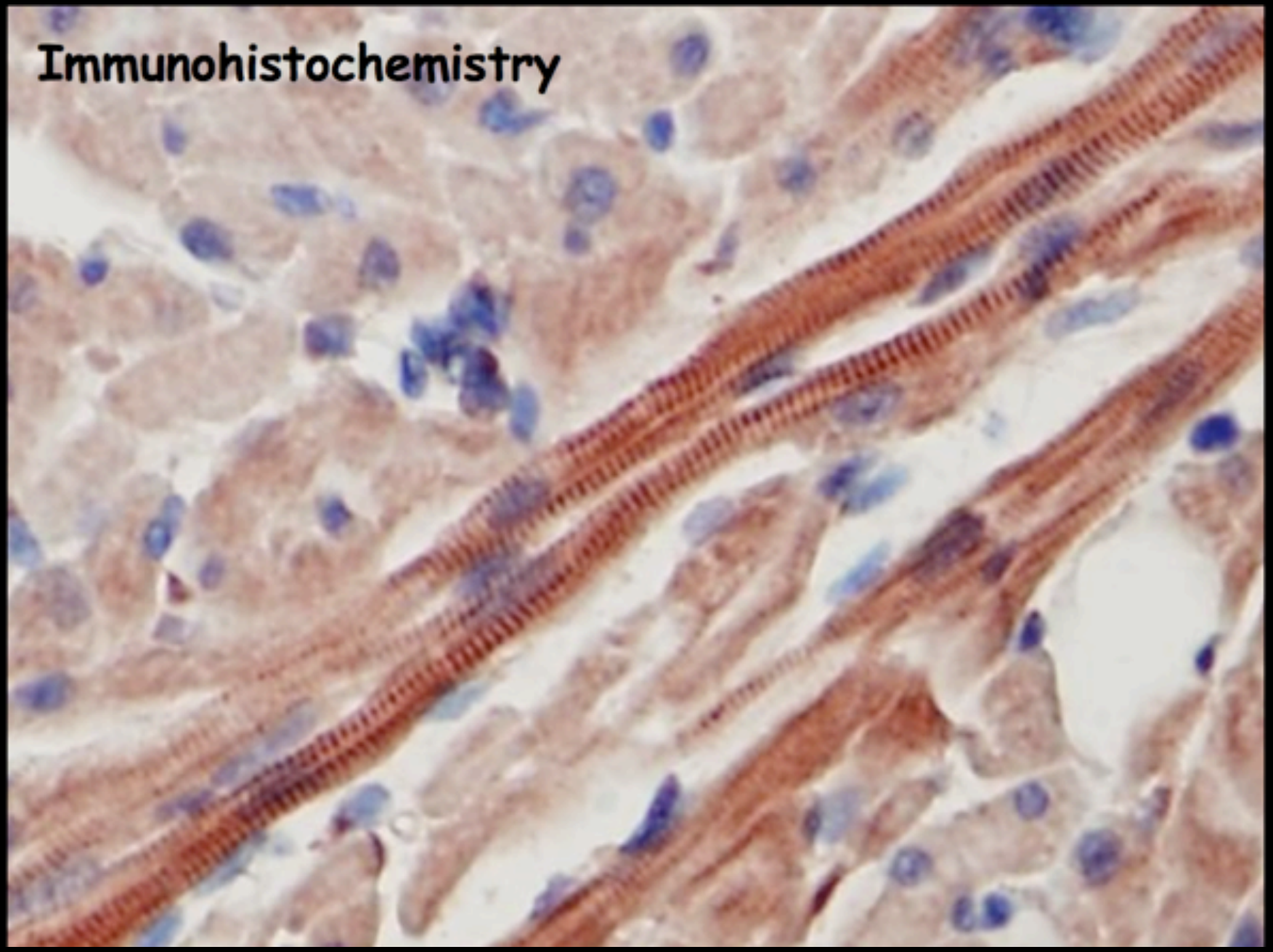
Immunofluorescence



Monoclonal antibodies directed against different antigens, which are conjugated to distinct fluorescent dyes can help in determining the localization of specific molecules within cells.

In the following figure, rat cardiac myocytes were stained with monoclonal antibodies specific for a cytoplasmic protein, and a cytoskeletal element, plus a blue dye that stain the nucleus.


A
B
C
D


Immunohistochemistry

Detection of Reed-Sternberg tumor cells in lymph nodes of two Hodgkin's Lymphoma patients using immunohistochemistry