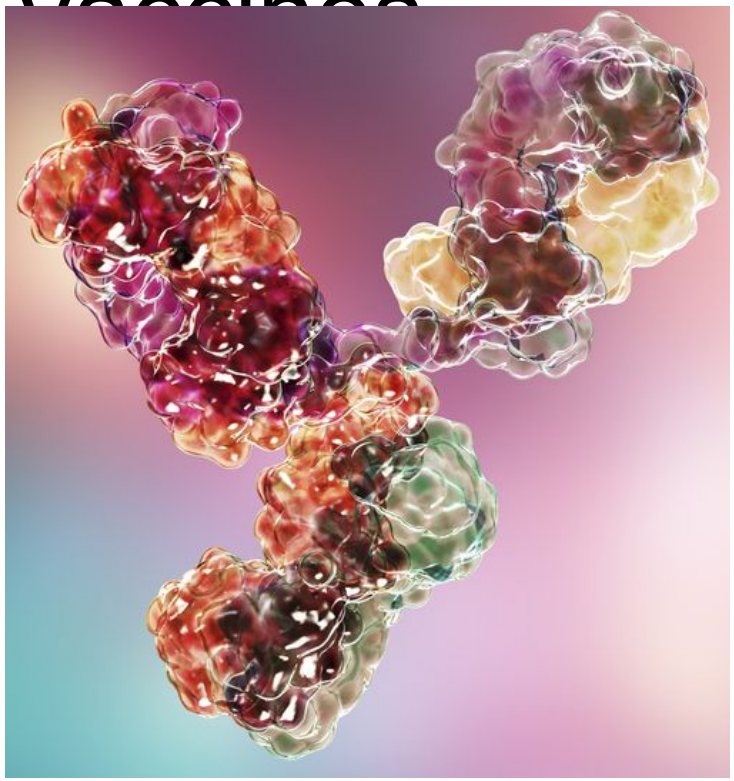
APPLICATIONS OF IMMUNOLOGY

Monoclonal Antibodies in Diagnostics & Therapeutics and Vaccines



BCH 4047 TD/TP.1
By

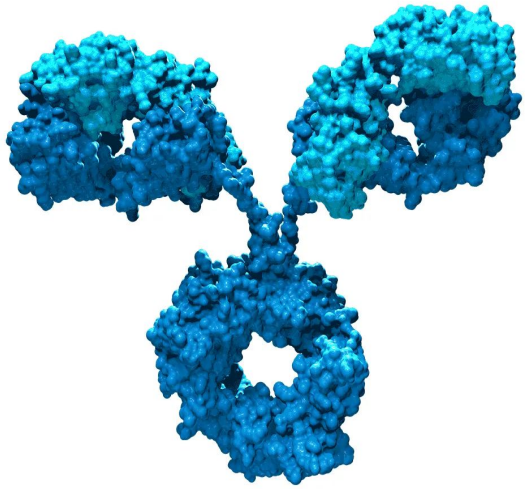
Prof Palmer Masumbe Netongo (PhD)

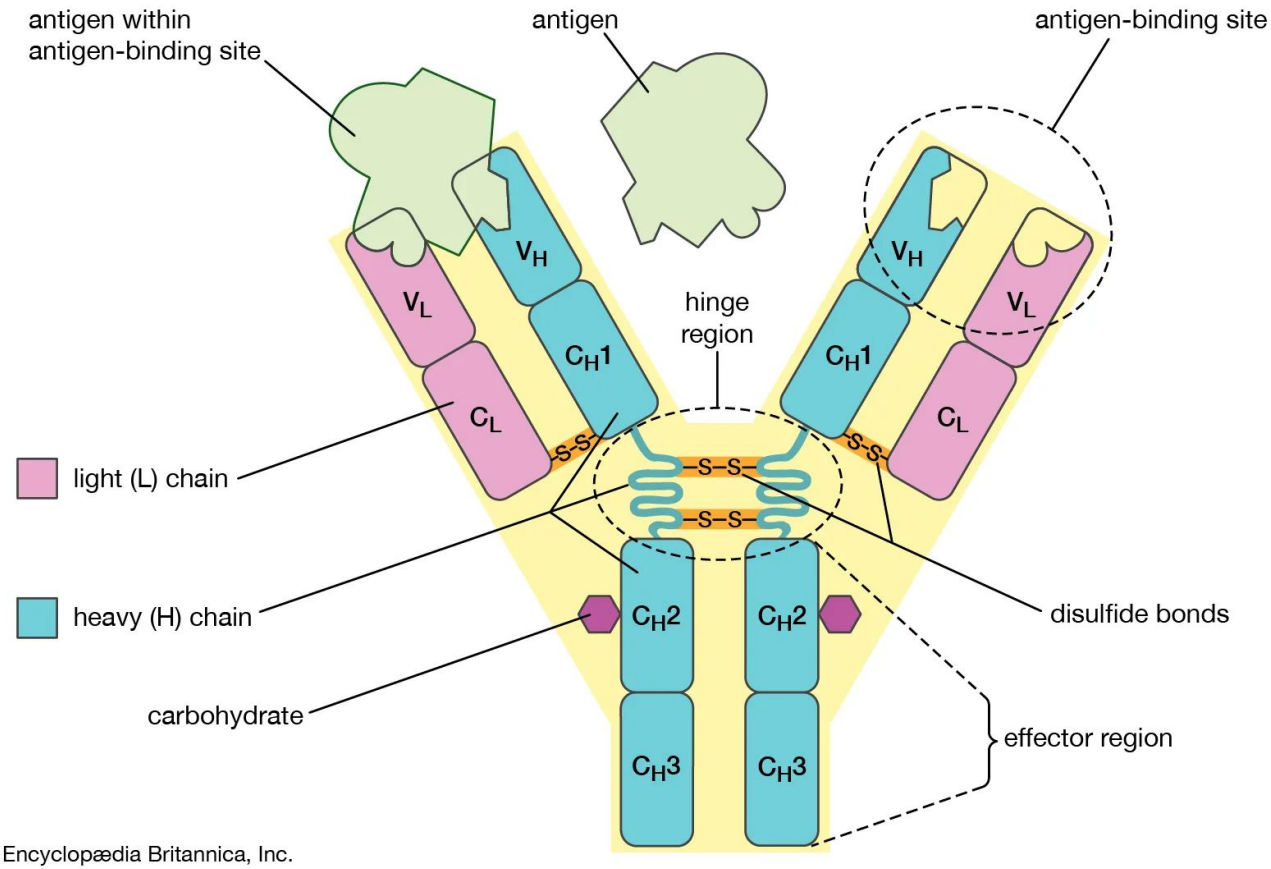
Immunological Techniques #1:

Monoclonal Antibody Production by Hybridoma Technology

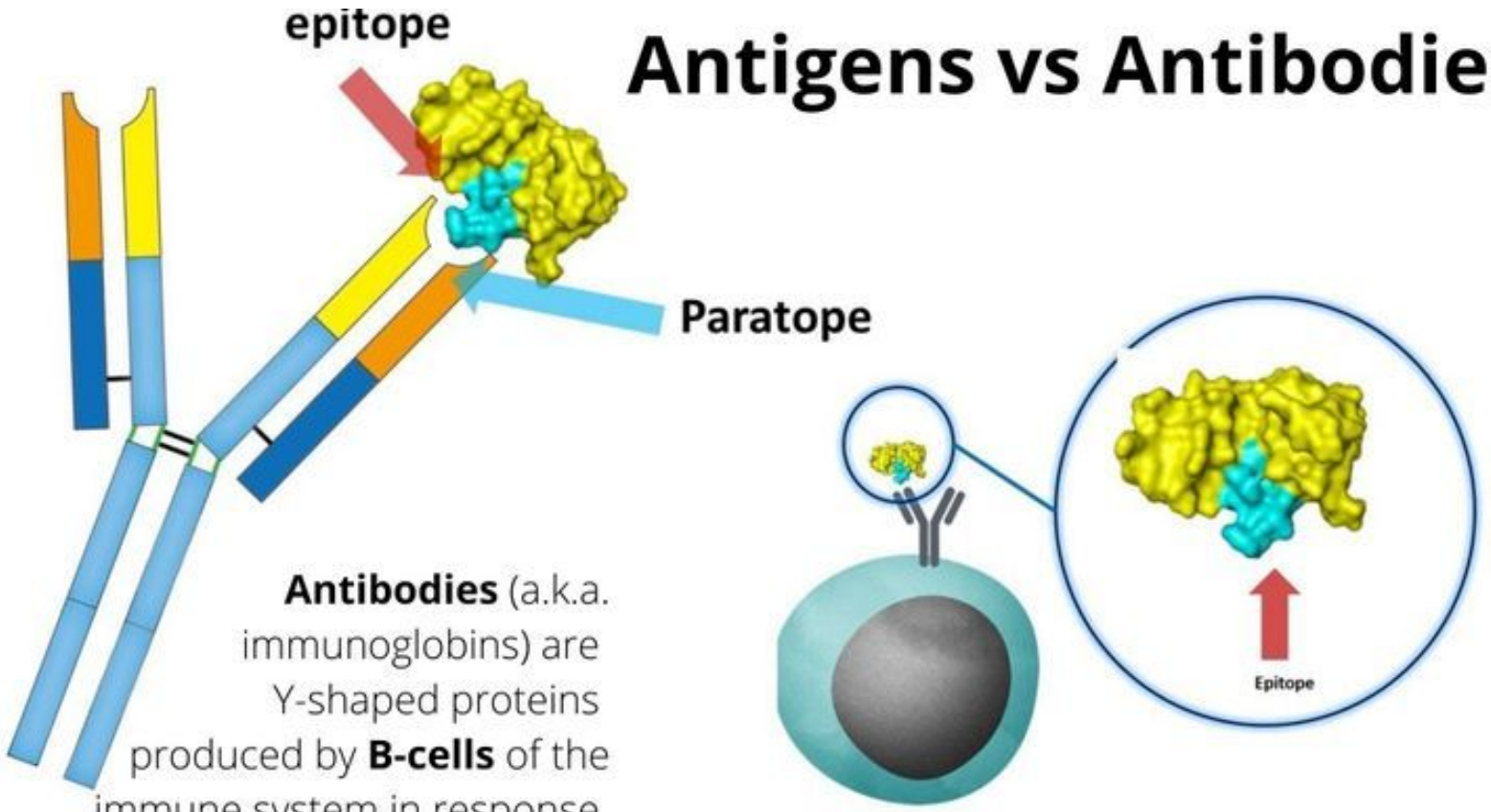
Recall antigens vs antibodies?

- What is an Antigen?
- What is an antibody?





It is all about Antigens & antibodies.



Antibodies (a.k.a. immunoglobins) are Y-shaped proteins produced by **B-cells** of the immune system in response to exposure to antigens. Each antibody contains a "**paratope**" which recognises a specific "**epitope**" on an antigen.

Antigens are molecules (i.e. in SARS-CoV-2) capable of triggering an immune response. Each antigen has distinct surface features, or "**epitopes**", resulting in specific responses

Learning Objectives

Student should be able to answer the following questions

1. Why study Monoclonal Antibody?
2. How are monoclonal antibodies different from polyclonal antibodies?
3. What are Monoclonal antibodies?
4. How are they produced?
5. What are the main applications of monoclonal antibodies?

Immunological Techniques #1:

Why study Monoclonal Antibody?

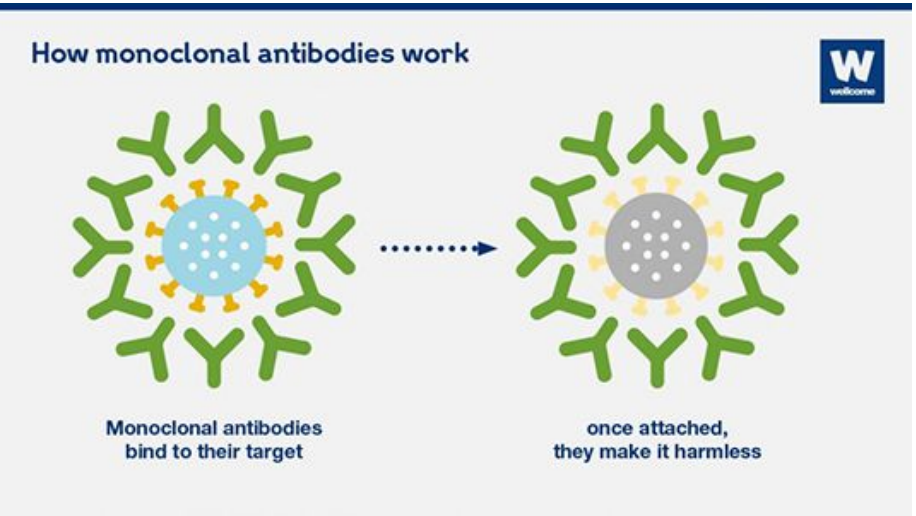
A. What is HIV and ARVs?

B. Why do people still die from HIV when they stop ARV treatment?

C. What are the alternatives to ARV treatment?

D. What else do we know today about mAbs?

E. What is omalizumab? (Home work)



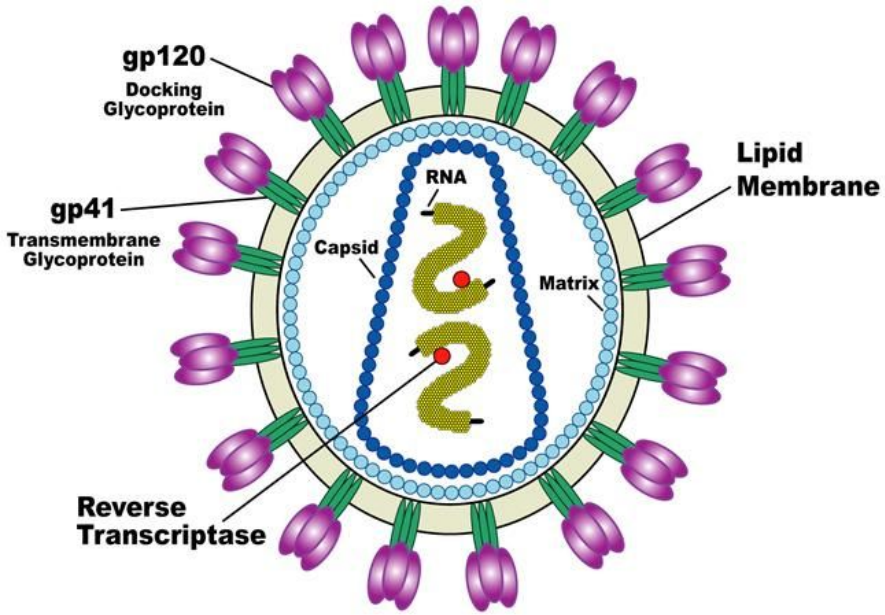
nature medicine
REVIEW ARTICLE
https://doi.org/10.1038/s41591-019-0412-8

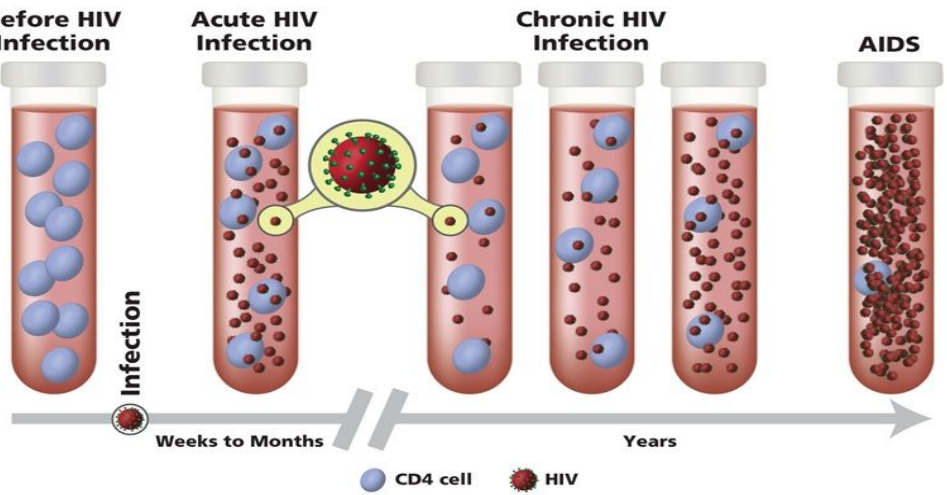
Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic

Marina Caskey[1]*, Florian Klein[2,3,4]* and Michel C. Nussenzweig[1,5]*

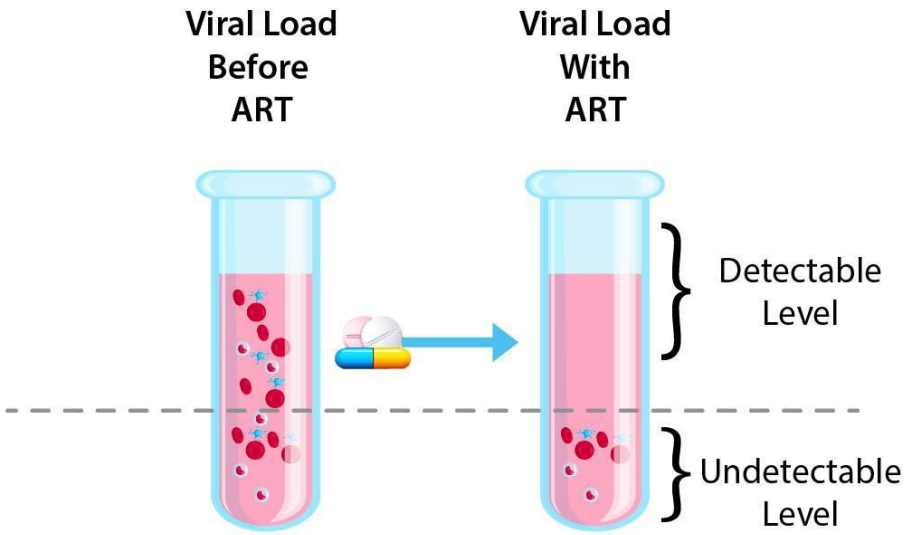
Combination anti-retroviral therapy (ART) has revolutionized the treatment and prevention of HIV-1 infection. Taken daily, ART prevents and suppresses the infection. However, ART interruption almost invariably leads to rebound viremia in infected individuals due to a long-lived latent reservoir of integrated proviruses. Therefore, ART must be administered on a life-long basis. Here we review recent preclinical and clinical studies suggesting that immunotherapy may be an alternative or an adjuvant to ART because, in addition to preventing new infections, anti-HIV-1 antibodies clear the virus, directly kill infected cells and produce immune complexes that can enhance host immunity to the virus.

A.What is HIV and ARVs?





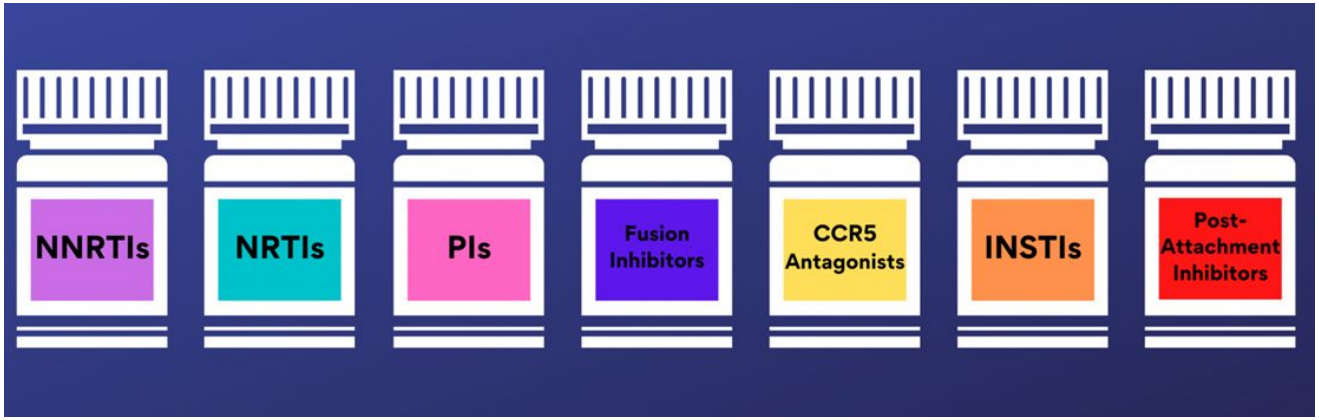
- ART- anti-retroviral therapy
- Combination anti-retroviral therapy (ART) has revolutionized the treatment and prevention of HIV-1 infection.
- Taken daily, ART prevents and suppresses the infection.



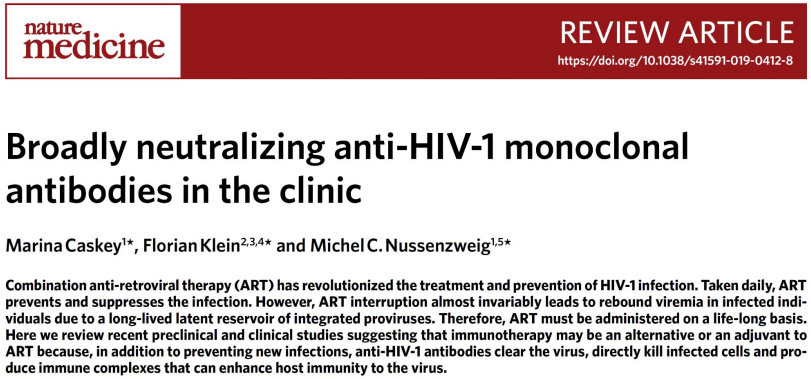
B. Why do people still die from HIV when they stop ARV treatment?

The Problem with ARVs:

- ART interruption almost invariably leads to rebound viremia in infected individuals due to a long-lived latent reservoir of integrated proviruses.
- Therefore, ART must be administered on a life-long basis.



C. What are the alternatives to ARV treatment?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Immunotherapy

- Review of recent preclinical and clinical studies suggest that **immunotherapy** may be an alternative or an adjuvant to ART because,
  - Anti-HIV-1 antibodies can **prevent new infections**, plus
  - **clear the virus**,
  - **directly kill infected cells** and
  - produce immune complexes that can **enhance host immunity to the virus.**

The immune response to HIV.

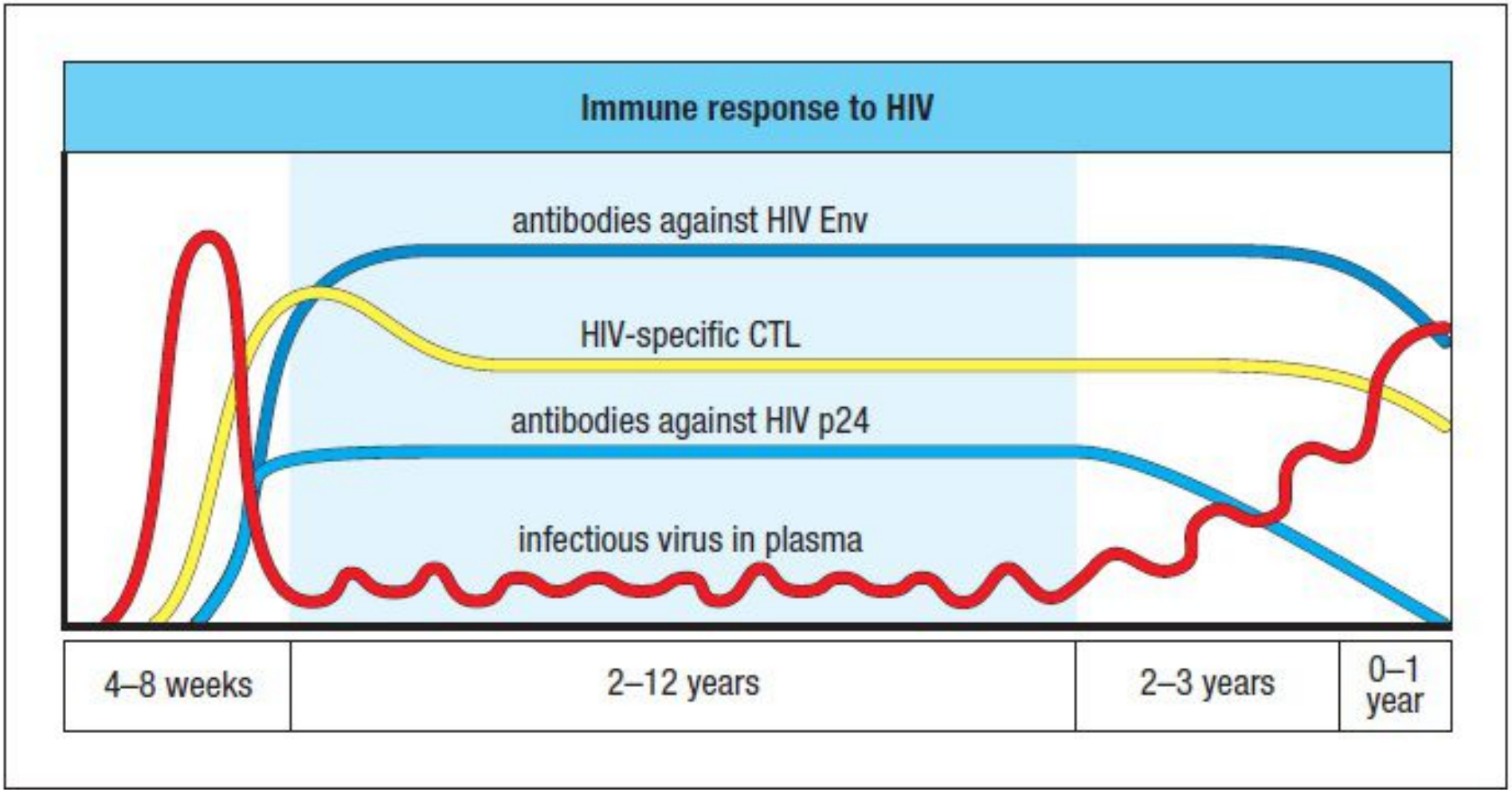
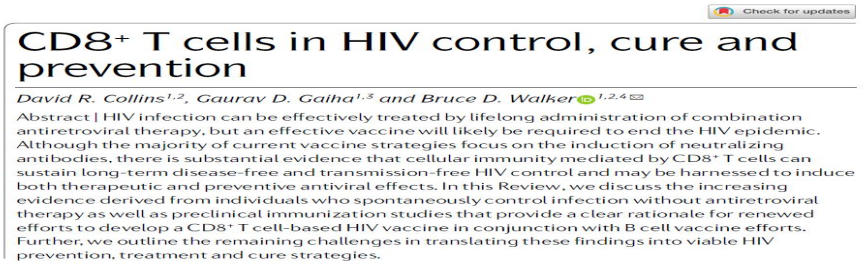


Fig. 13.41 Janeway immunobiology 10th ed.

What else do we know today about mAbs?

- Cytotoxic CD8 T cells are clinically important for the control of HIV-infected cells?
  - Evidence for the clinical importance of the control of HIV-infected cells by cytotoxic CD8 T cells comes from studies relating the numbers and activity of CD8 T cells to viral load.
  - There is also direct evidence from experiments in macaques infected with SIV that cytotoxic CD8 T cells control retrovirus infected cells;
  - treatment of infected animals with monoclonal antibodies that remove CD8 T cells is rapidly followed by a large increase in viral load.

Nat Rev Immunol. 2020; 20(8): 471–482.
Published online 2020 Feb 12. doi: 10.1038/s41577-020-0274-9


REVIEWS

Check for updates

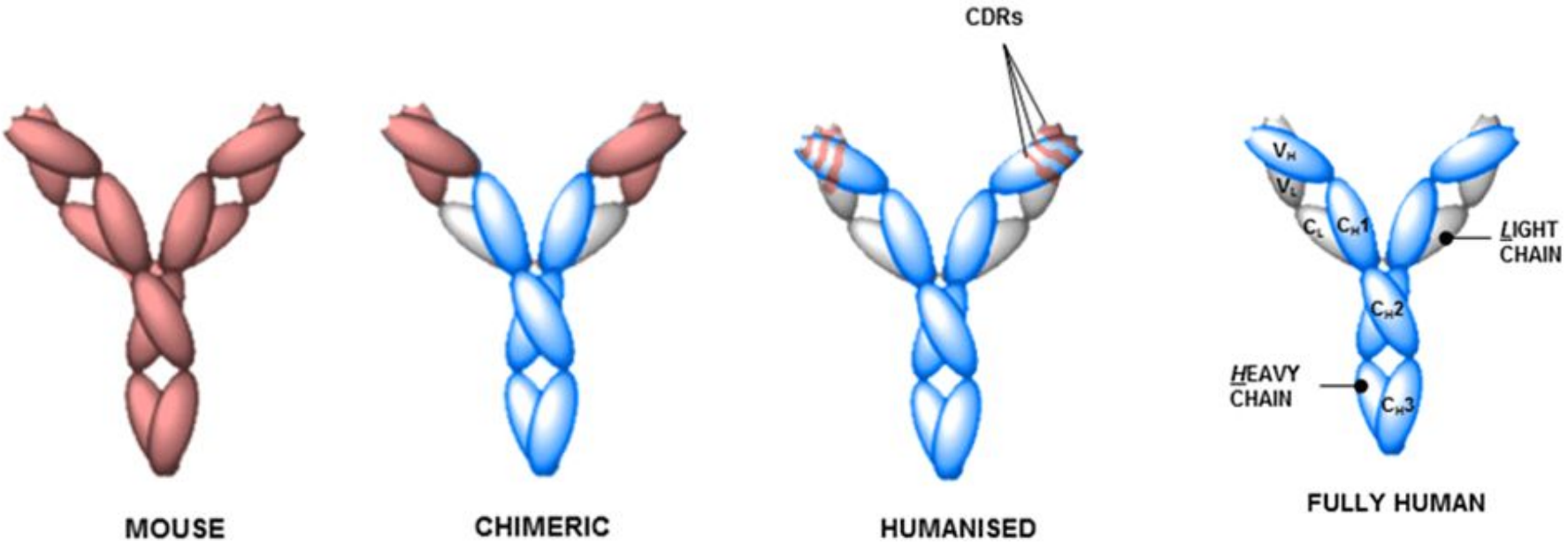
CD8⁺ T cells in HIV control, cure and prevention

David R. Collins[1,2], Gaurav D. Gaiha[1,3] and Bruce D. Walker[1,2,4]

Abstract | HIV infection can be effectively treated by lifelong administration of combination antiretroviral therapy, but an effective vaccine will likely be required to end the HIV epidemic. Although the majority of current vaccine strategies focus on the induction of neutralizing antibodies, there is substantial evidence that cellular immunity mediated by CD8⁺ T cells can sustain long-term disease-free and transmission-free HIV control and may be harnessed to induce both therapeutic and preventive antiviral effects. In this Review, we discuss the increasing evidence derived from individuals who spontaneously control infection without antiretroviral therapy as well as preclinical immunization studies that provide a clear rationale for renewed efforts to develop a CD8⁺ T cell-based HIV vaccine in conjunction with B cell vaccine efforts. Further, we outline the remaining challenges in translating these findings into viable HIV prevention, treatment and cure strategies.

Modified Antibodies used today in diagnostics and treatment

omab **-ximab** **-zumab** **-umab**



MAbs Immunosuppressive drugs

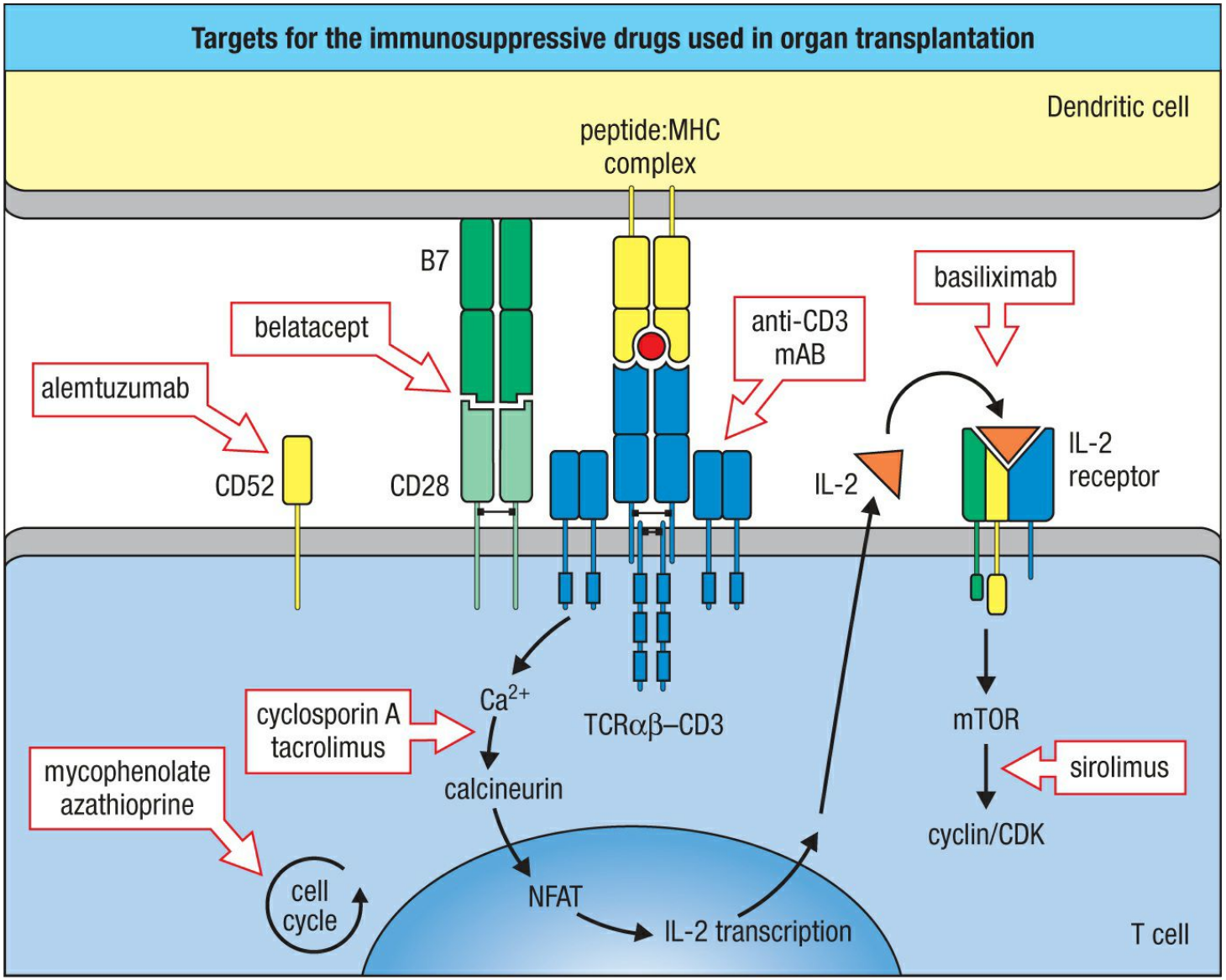


Fig. 13.41 Janeway immunobiology 10th ed.

MAbs that recognize tumor-specific antigens have been used to help eliminate tumors.

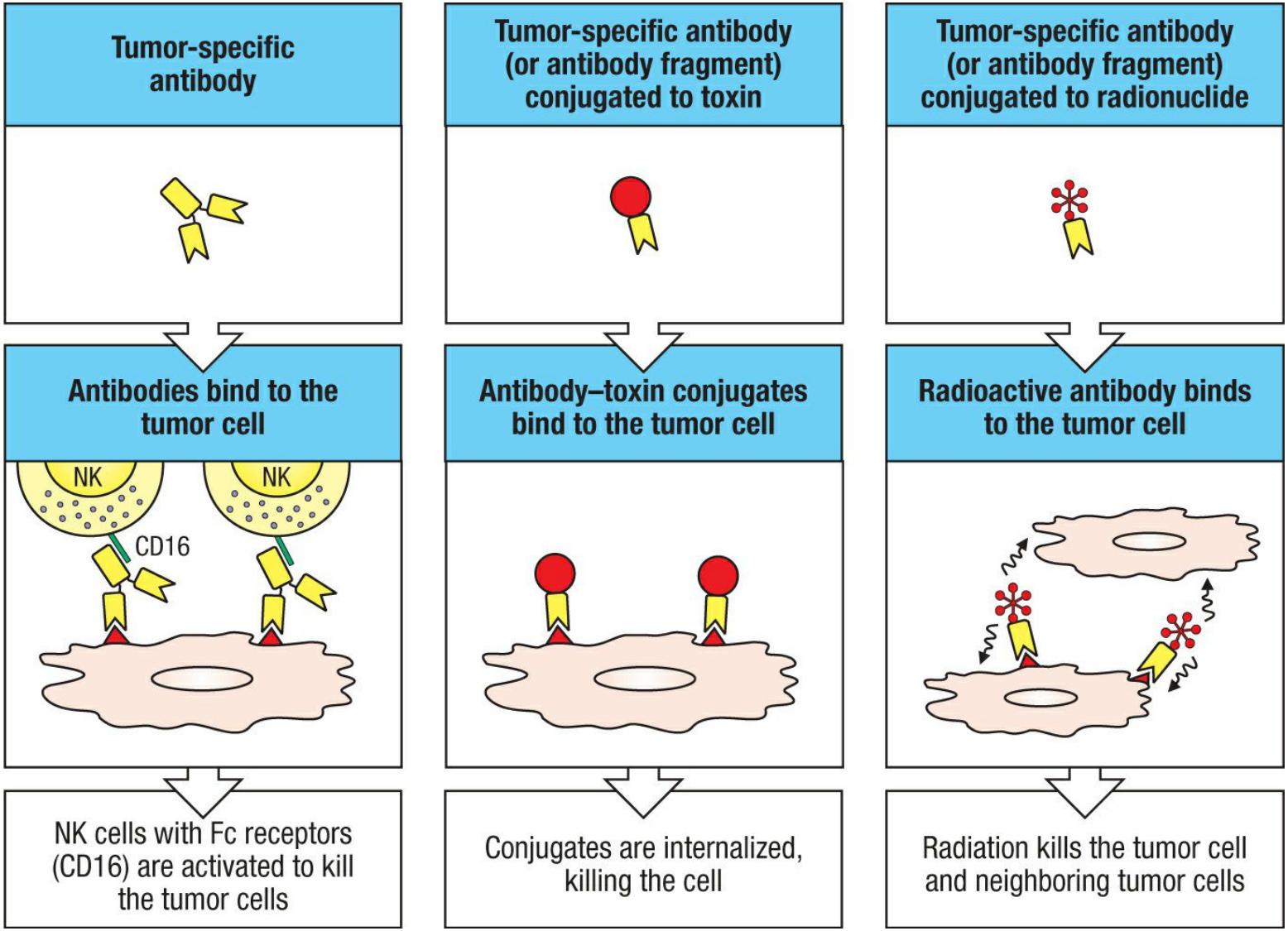
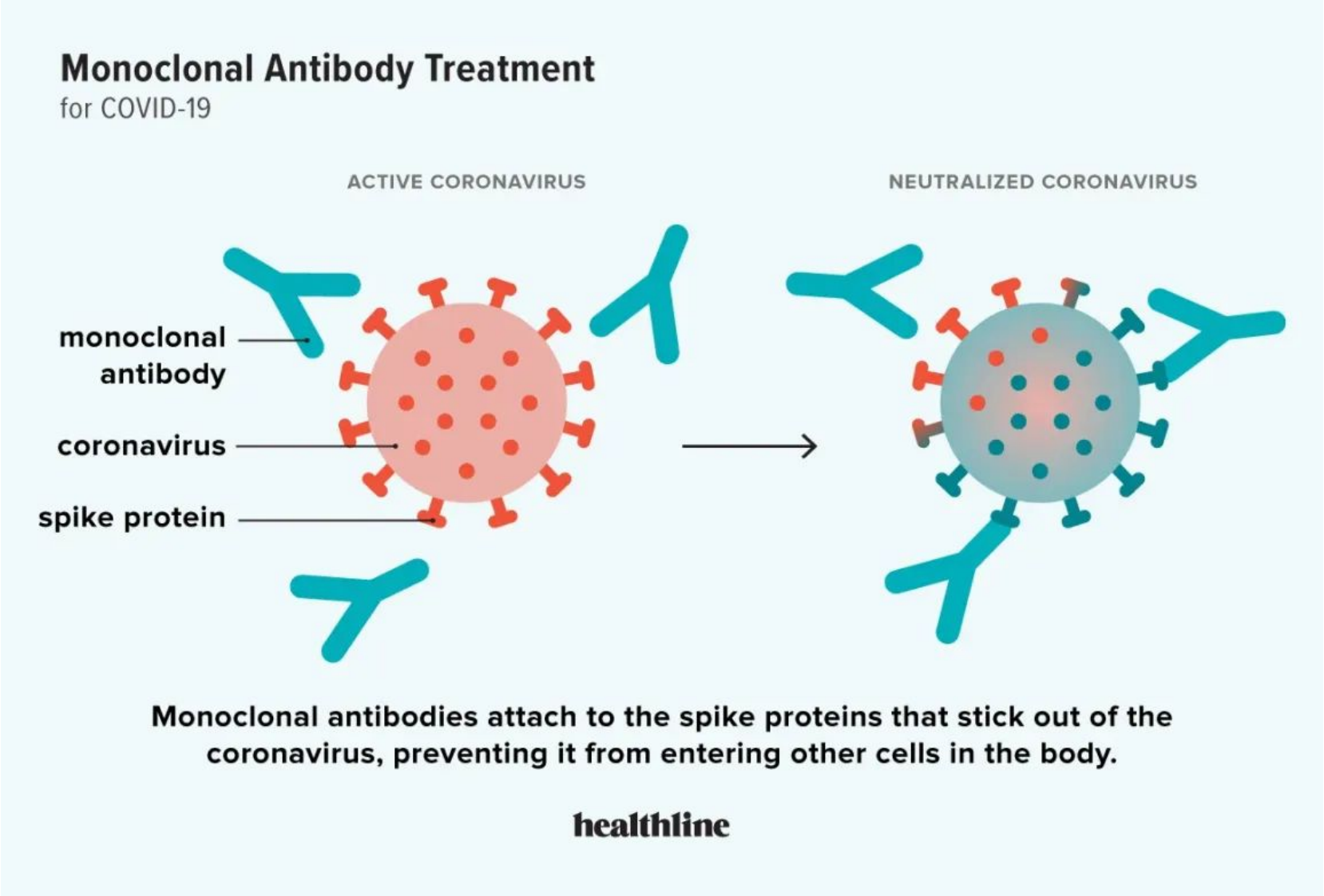


Fig. 16.22, Janeway immunobiology 10th ed.

MAbs were critical in treating patients with Covid-19 by anti-spike neutralization.



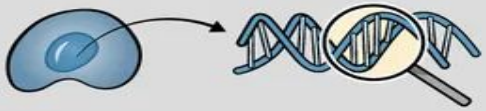
A bridge to vaccines: Monoclonal antibodies could save lives and slow the spread of the coronavirus

How to make **monoclonal antibodies**

1. Take blood from a person who recovered from COVID-19

2. Use "bait" molecules ⬡ to fish out the B cells that produce antibodies for a key portion of the novel coronavirus spike protein and block infection

3. Decipher the DNA for those antibodies



4. Insert that DNA into cells that mass-produce the antibodies.

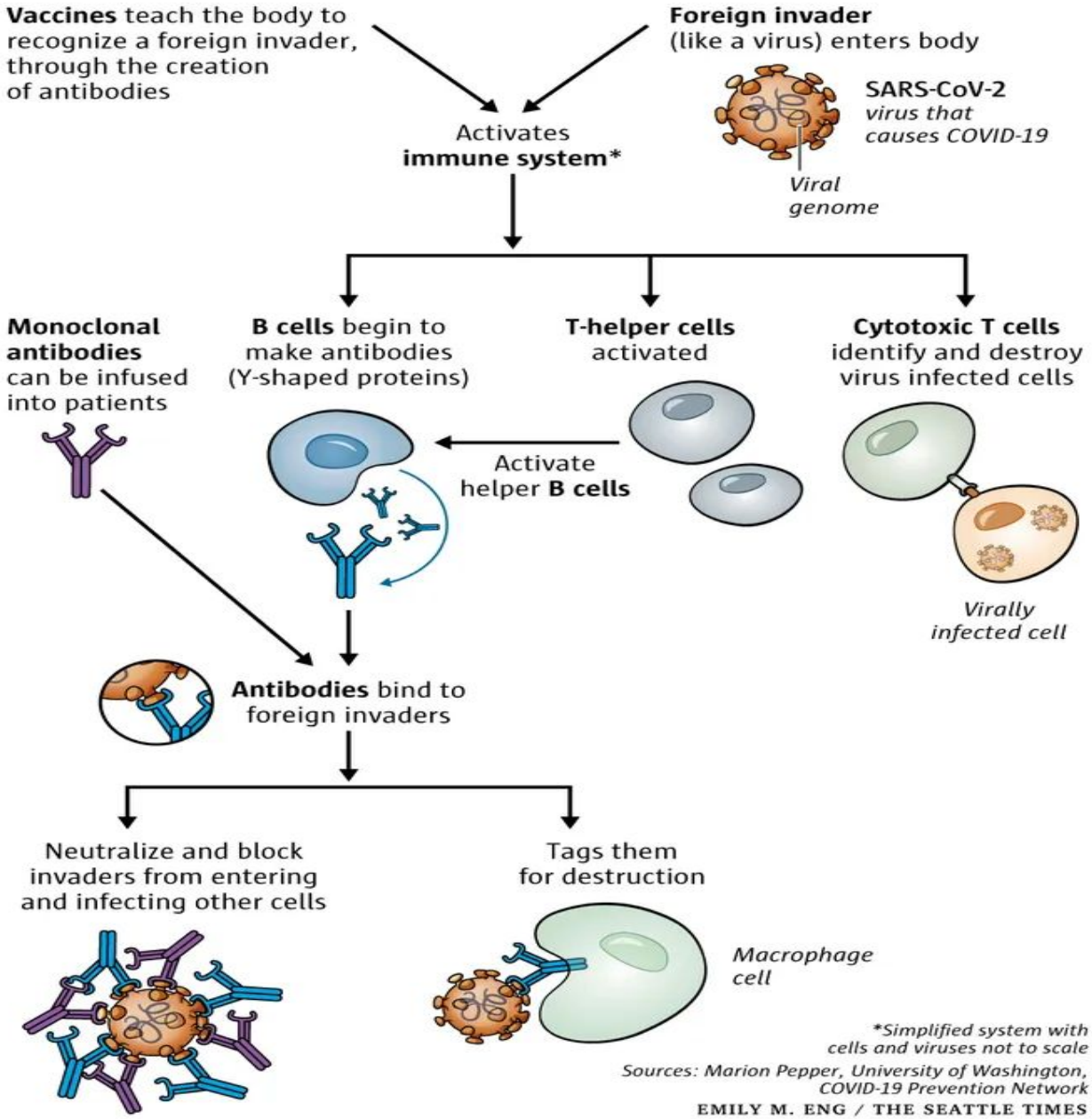
Potential benefits:

- Prevention option before a vaccine is available
- Provide immediate protection or treatment for those exposed
- Benefits to people who cannot develop or maintain an adequate immune response after vaccination

Monoclonal antibody limitations:

- Protection is short-lived
- The drugs are expensive

HOW VACCINES AND MONOCLONAL ANTIBODIES WORK



*Simplified system with cells and viruses not to scale

Sources: Marion Pepper, University of Washington, COVID-19 Prevention Network

EMILY M. ENG / THE SEATTLE TIMES

How are monoclonal antibodies different from polyclonal antibodies?

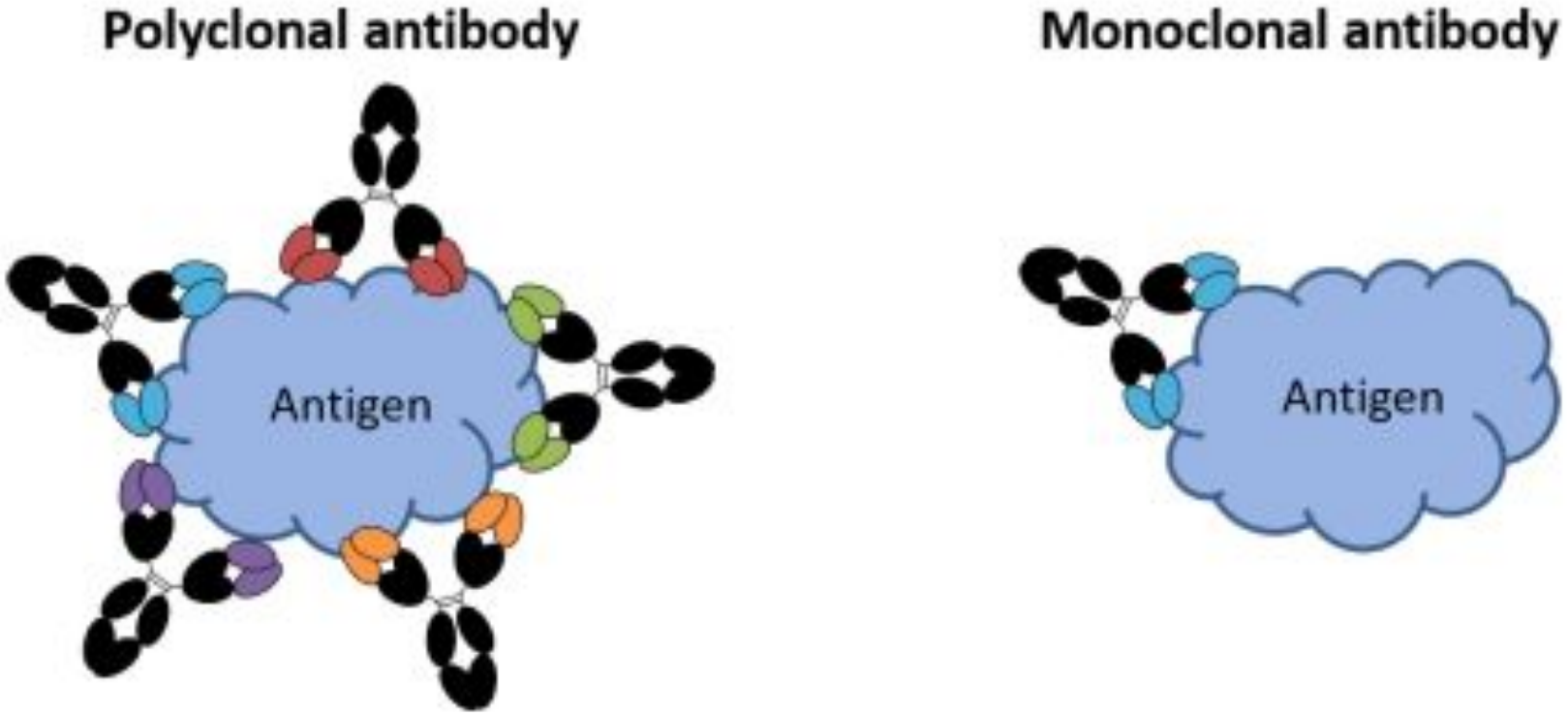
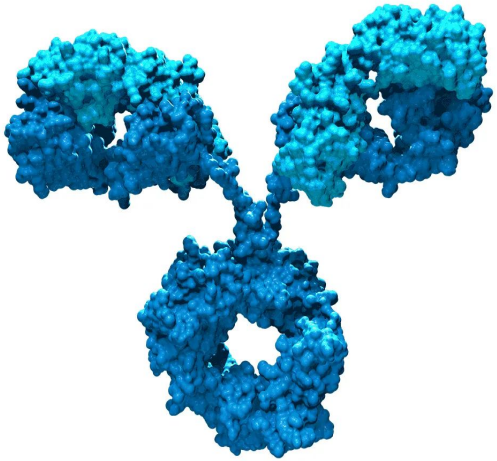
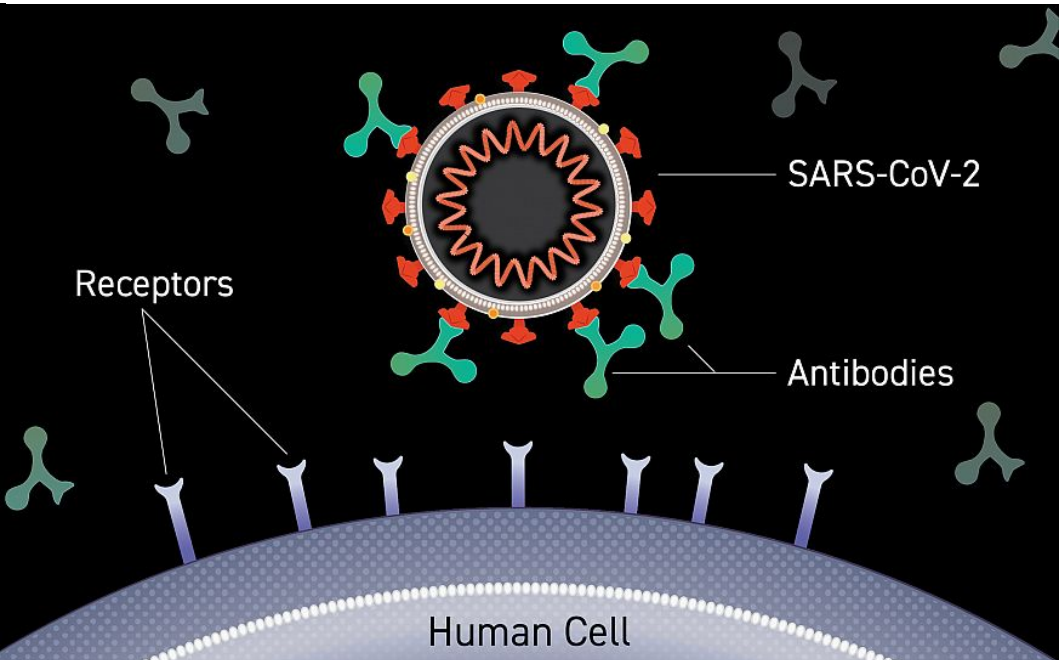
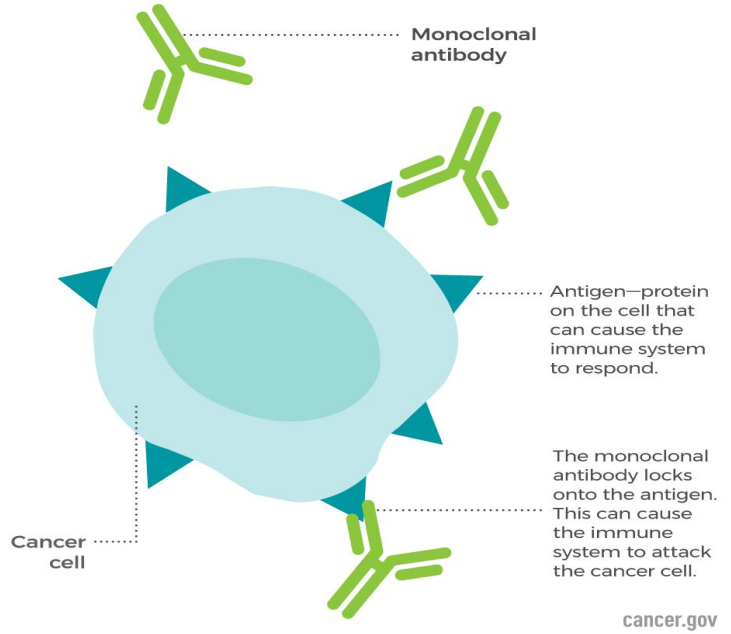


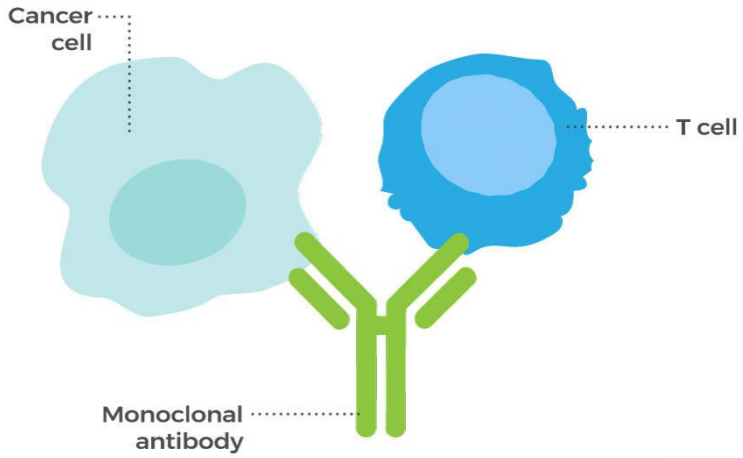
Fig 1. Monoclonal and Polyclonal Antibodies. The polyclonal antibody can bind to a single antigen, but can bind to different forms of it. The monoclonal antibody can only bind to a specific type of antigen.

What are Monoclonal antibodies?

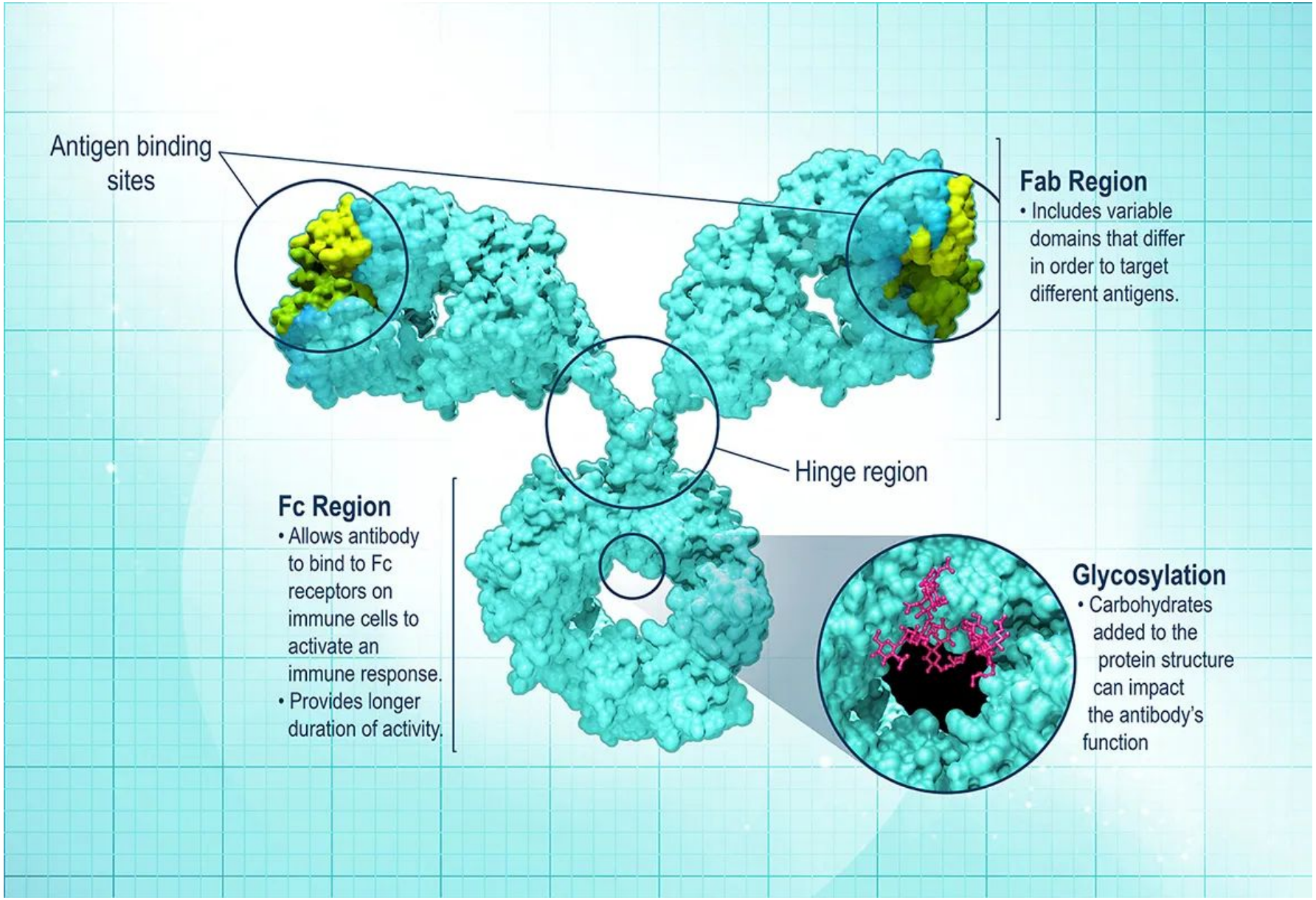




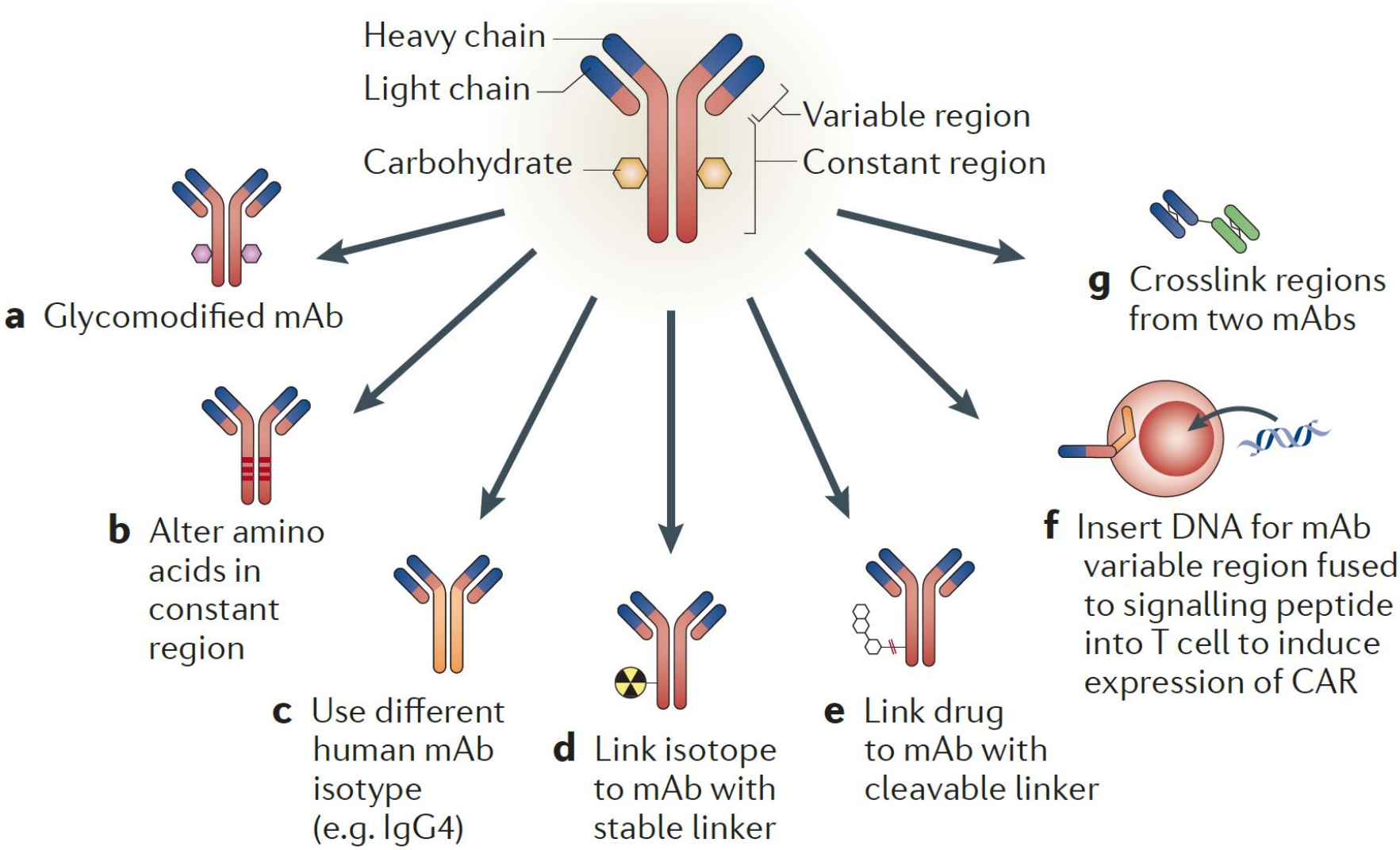




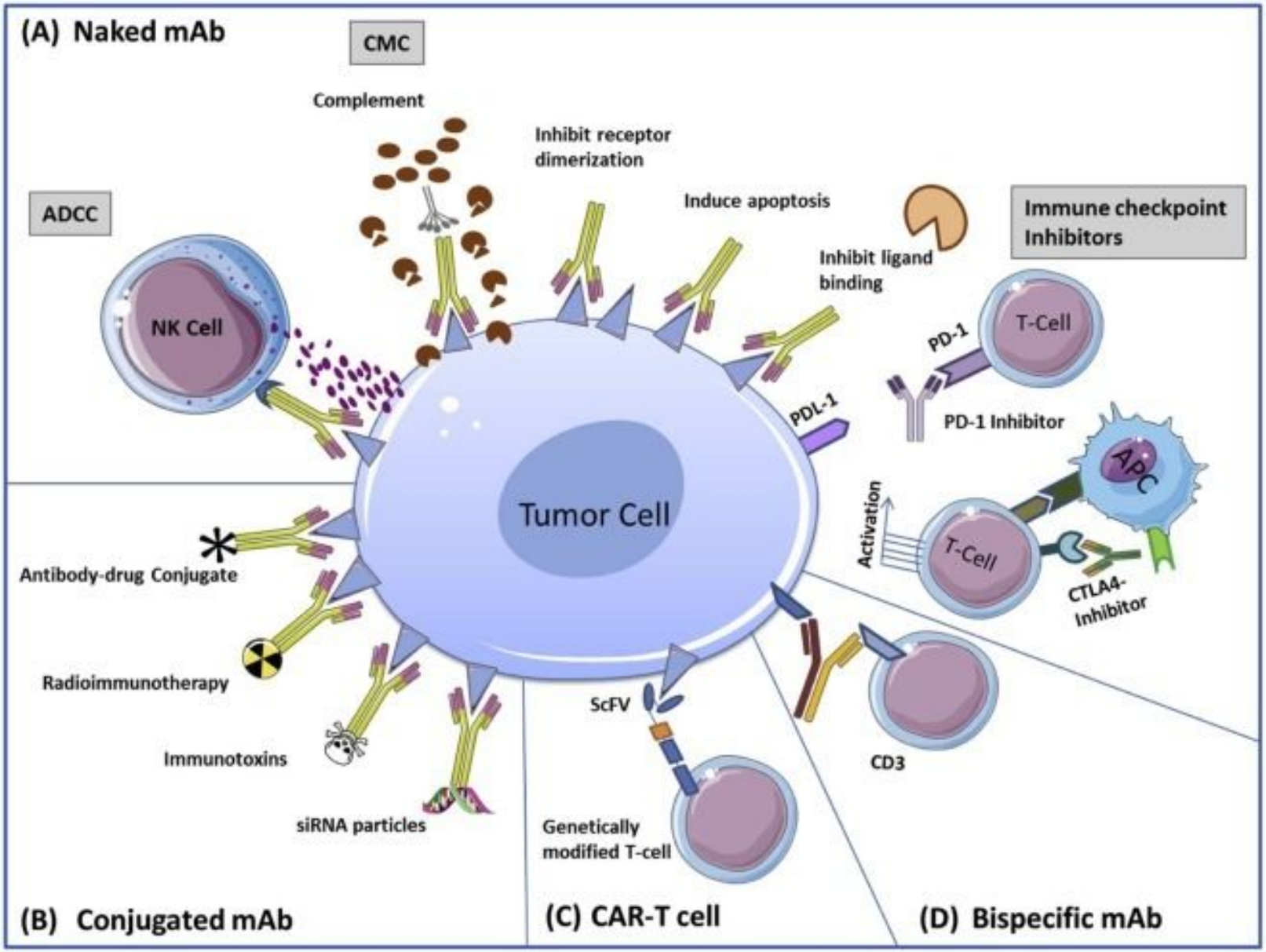
What are Monoclonal antibodies?



Modifying monoclonal antibody structure.



Mechanism of action of mAb therapy.



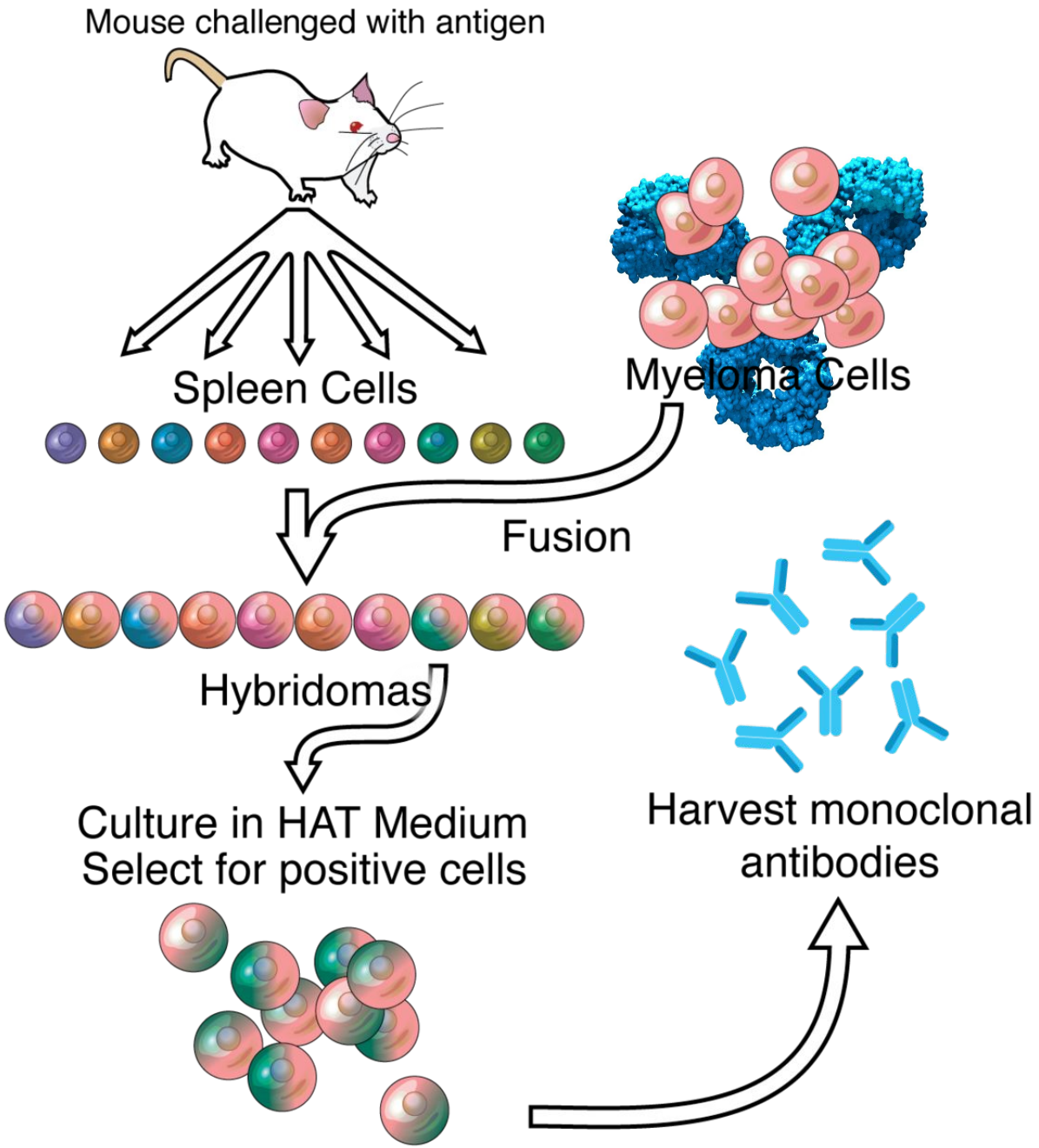
Immunological Techniques #1:

Monoclonal Antibody Production by Hybridoma Technology

What are Monoclonal antibodies?

- Monoclonal antibody is a type of protein that is made in the laboratory and can bind to certain targets in the body, such as antigens on the surface of cancer cells or infected cells.
- There are many kinds of monoclonal antibodies, and each monoclonal antibody is made so that it binds to only one antigen and more specifically to one epitope on an antigen.

Monoclonal Antibody?



How are mAbs produced?

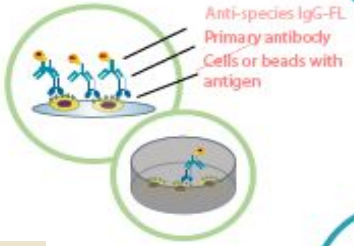
Monoclonal Antibody Production

Immunization of mice & isolation of splenocytes
Mice are immunized with an antigen and later their blood is screened for antibody production. The antibody-producing splenocytes are then isolated for *in vitro* hybridoma production.

Preparation of myeloma cells
Myeloma cells are immortalized cells that, once fused with spleen cells, can result in a hybridoma capable of unlimited growth. Myeloma cells are prepared for fusion.

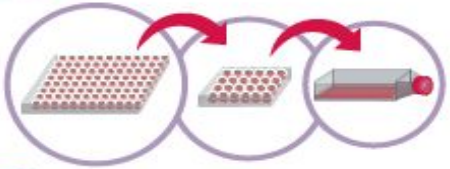
Fusion
Myeloma cells and isolated splenocytes are fused together to form hybridomas in the presence of polyethylene glycol (PEG), which causes cell membranes to fuse.

Clone screening and picking
Clones are screened and selected on the basis of antigen specificity and immunoglobulin class.

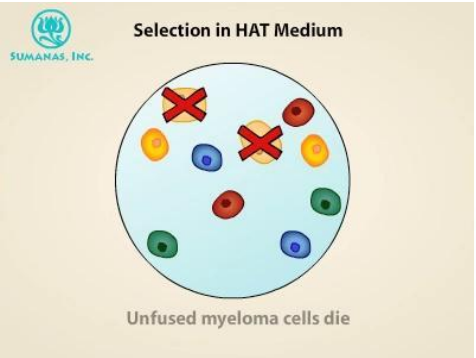


Functional characterization
Confirm, validate, and characterize (e.g. ELISA) each potentially high-producing colony.

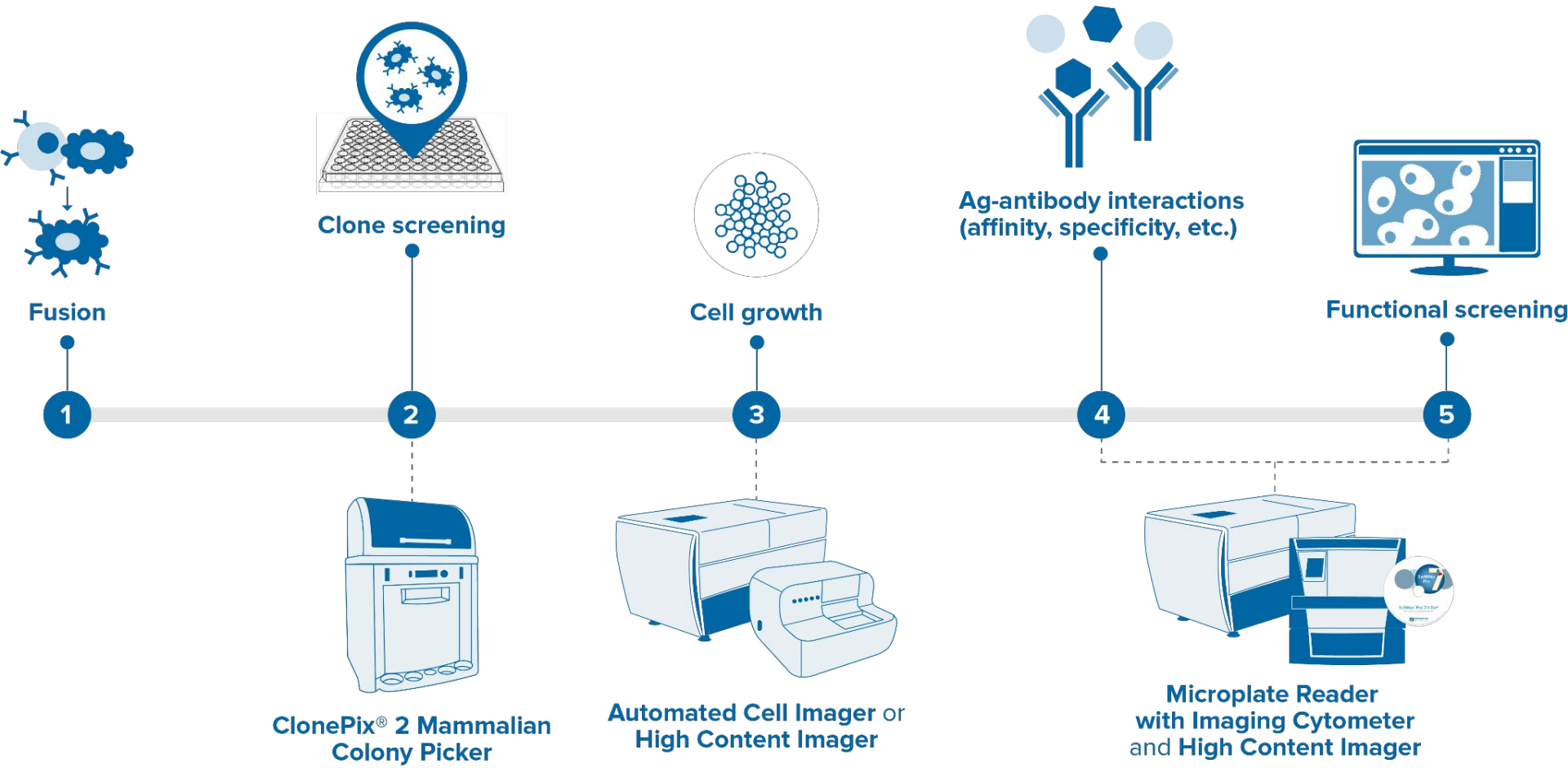
Scale up and wean
Scale up clones producing desired antibodies and wean off selection agent(s).



Expansion
Expand clones producing desired antibodies (e.g. bioreactors or large flasks).



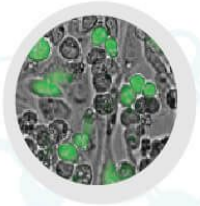
Automated mAbs production.



What are the main applications of mAbs?

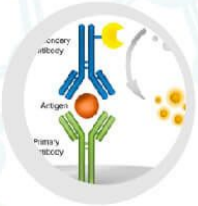
Why did President Trump spend just 3 days in hospital when he contracted Covid-19?

CELL COUNT / CELL VIABILITY
- Live/Dead (EarlyTox) Staining
- MTT Assay
- Transfection Efficiency
- Cell Proliferation (Cell Growth)
- CellTiter-Glo

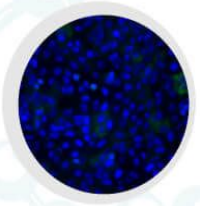


CELL LINE DEVELOPMENT
- Colony Picking
- Monoclonality Verification
- Cell Sorting
- Cell Line Knock-Ins/Outs

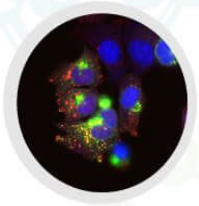
BINDING AFFINITY
- ELISA (IgG/IgM)
- Cell-based Binding
- HTRF
- AlphaScreen/AlphaLISA



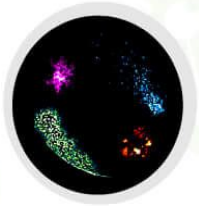
PROTEIN/NUCLEIC ACID QUANTIFICATION (NON-SPECIFIC)
- BCA
- Bradford Assay
- RNA/DNA Quantification



VIRAL NEUTRALIZATION
- PRNT
- VRNT
- MicroNeutralization

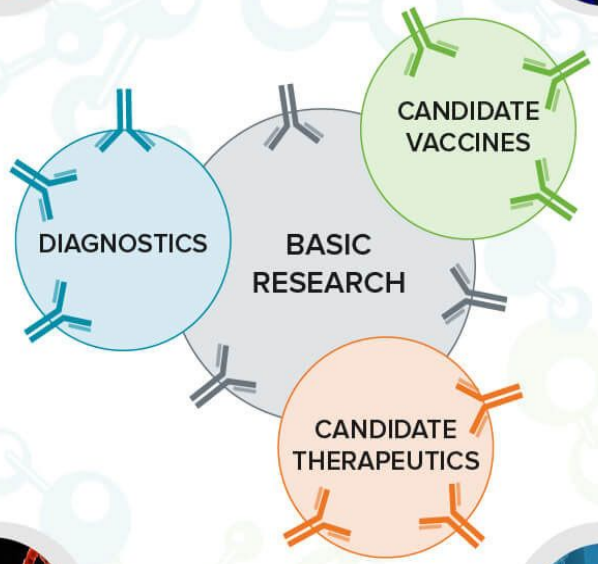


VIRAL PATHOGENESIS
- Internalization
- Cleavage-based Reporters
- Reporter Gene Assays
- Second Messenger Assays
- Protein Modification Assays

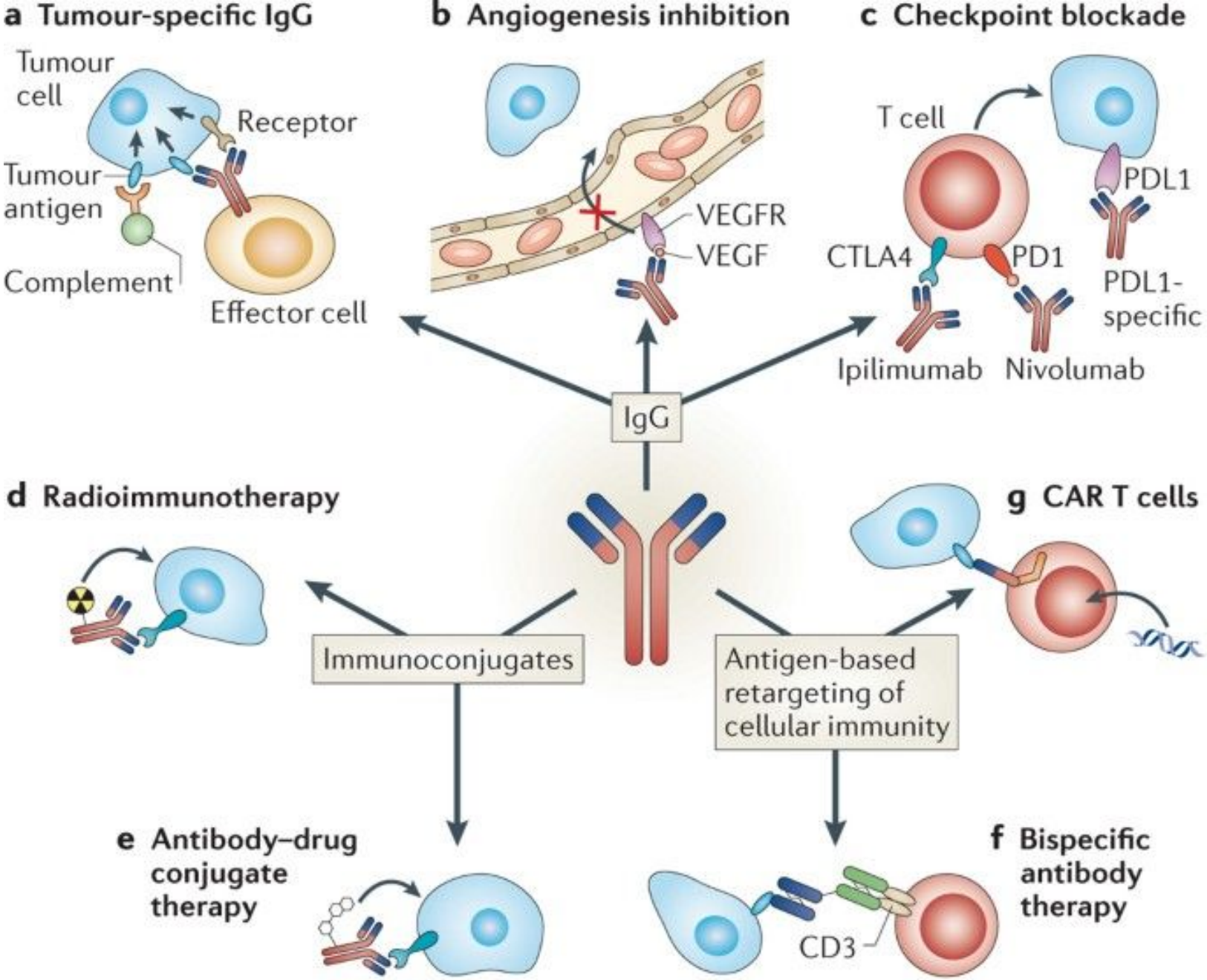


VIRAL TITER
- Plaque Assay
- Focus Forming Assay
- Endpoint Dilution

PROTEIN/NUCLEIC ACID QUANTIFICATION (SPECIFIC)
- ELISA (Cytokine Secretion)
- Western Blot
- AlphaScreen / AlphaLISA
- Fluorescence Polarization ImmunoAssay
- HTRF

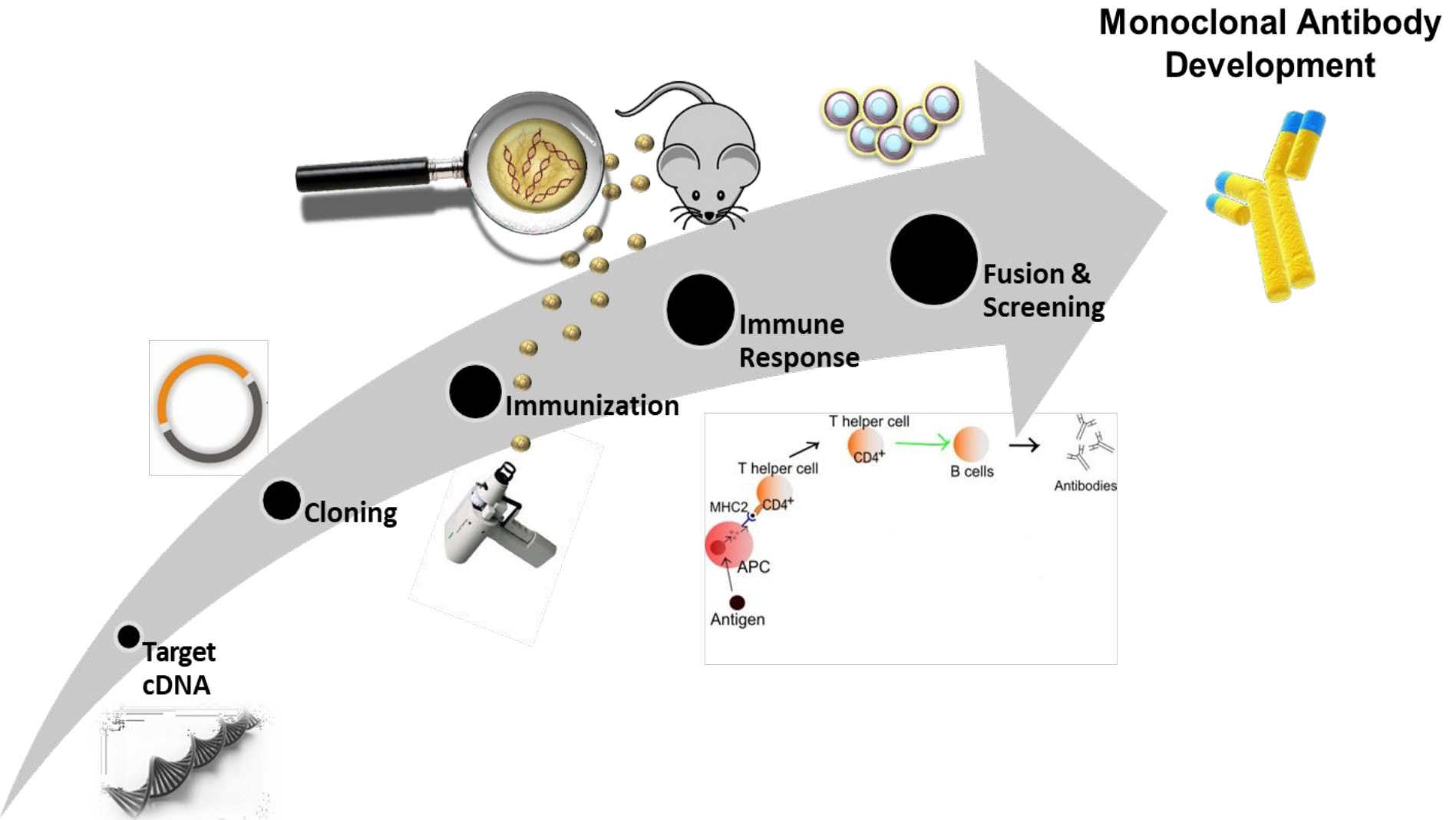


Applications of mAbs.



Alternative method of monoclonal antibody production

DNA Immunization for Antibody Generation



Disadvantages of mAbs

- Genetic drift is one concern when using hybridomas to produce monoclonal antibodies. Genetic drift refers to changes in the nucleic acid sequence of the antibody-encoding genes over time as the hybridoma cells divide. These changes can alter the antibody that is actually produced by the hybridoma, meaning that there will be changes between lots over time.
- Specifically, the antigen-binding sites of the antibody - the paratopes - may change and impact the specificity and avidity of the antibodies produced by the hybridoma.
- Any genetic drive can be identified by sequencing the heavy and light chains.
- To circumvent any possible genetic drift, scientists can freeze hybridoma cells before culturing or they can clone the genes from the hybridoma into a plasmid to create a recombinant antibody.

APPLICATIONS OF IMMUNOLOGY

Monoclonal Antibodies in Diagnostics

BCH 4047 TD/TP. #2

By

Dr Palmer Masumbe Netongo (PhD)