BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biochemistry

Course Title : Immunology
Course Code : BC301CR

Unit-IV
Vaccines

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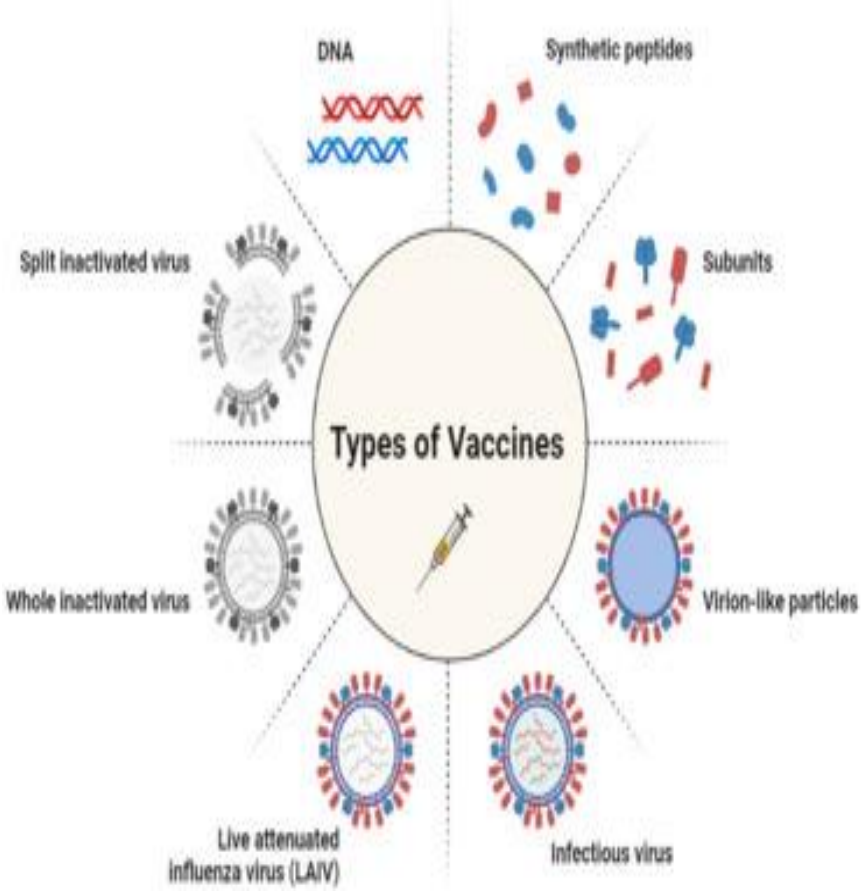
Vaccines killed, attenuated –toxoids, recombinant vectors, DNA

VACCINE:

- Vaccine is a suspension of weakened, killed, or fragmented microorganisms or toxins or other biological preparation, such as those consisting of antibodies, lymphocytes, or mRNA, that is administered primarily to prevent disease.

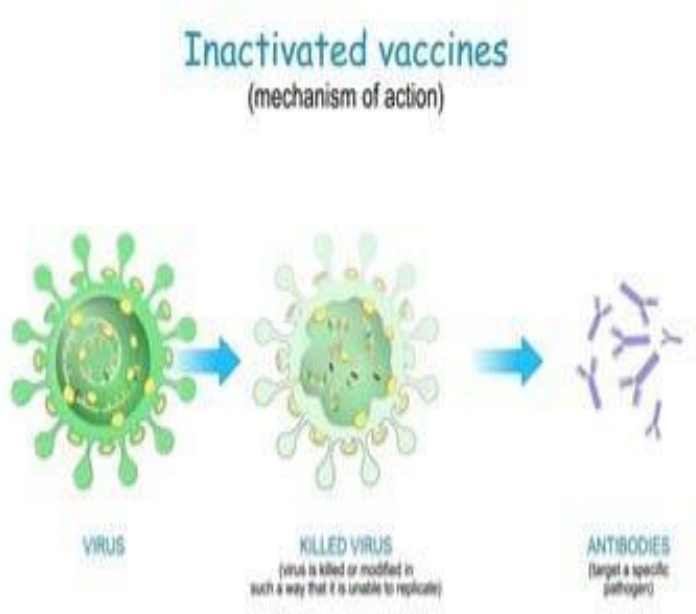
TYPES OF VACCINE:

- There are several types of vaccines, including:
- Inactivated vaccines
- Live-attenuated vaccines Messenger RNA (mRNA) vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines
- Viral vector vaccines



INACTIVATED OR DEAD VACCINES:

- The disease-causing pathogen is killed or inactivated, usually through a thermal (application of high temperature) or chemical (formalin etc.) process.
- Such vaccines, when administered, elicit a robust immune response that mimics most of the responses seen during an infection.
- Examples:
- Typhoid vaccine Influenza vaccine
- Salk polio vaccine Hepatitis A vaccine



PRODUCTION:

- Isolation: Isolate the pathogen from a clinical sample.
- 2. Cultivation: Grow the pathogen in large quantities.
- 3. Inactivation: Kill the pathogen using methods are Heat, Formaldehyde, Radiation , Detergents.
- 4. Purification: Remove impurities and contaminants.
- 5. Formulation: Prepare the final vaccine product (e.g., liquid, freeze-dried)
- 6. Quality Control: Test for safety, potency, and purity.

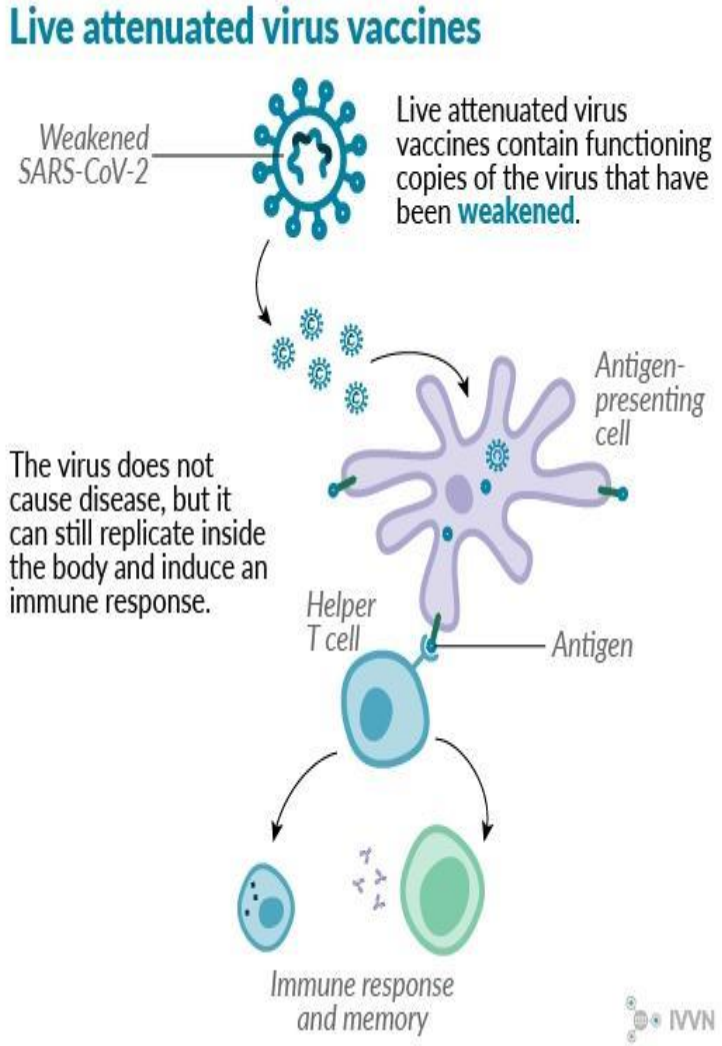
METHODS OF INACTIVATION:

- Heat inactivation
- Formaldehyde inactivation Radiation inactivation (e.g., gamma radiation)
- Detergent inactivation

- **Advantages**:
- No risk of reversion to virulence
- Suitable for immunocompromised individuals
- Easy to produce and store
- **Disadvantages**:
- May not induce strong immune response
- Require booster shots
- May contain residual toxins

LIVE ATTENUATED VACCINES

- Pathogens like virus or bacteria are weakened by genetic manipulations to limit its growth and thus do not cause disease to the host.
- In some modified versions of live vaccine an organism that is related to the pathogen is used that naturally grows poorly in humans.
- The weakened pathogen generates a broad immune response in the host similar to that shown by an infected individual with a natural pathogen.
- Examples: Oral Sabin polio vaccine ,MRV Vaccine (Measles, Mumps, Rubella, and Varicella) ,Nasal influenza vaccine



PRODUCTION:

- Isolation: Isolate the pathogen from a clinical sample
- Attenuation: Weaken the pathogen through various methods (e.g., passage through cell culture, mutagenesis)
- Characterization: Confirm the attenuated pathogen’s identity and stability
- Cultivation: Grow the attenuated pathogen in large quanti
- Harvesting: Collect the attenuated pathogens from the culture
- Purification: Remove impurities and contaminants
- Formulation: Prepare the final vaccine product (e.g., freeze-dried, liquid)
- Quality Control: Test for safety, potency, and purity

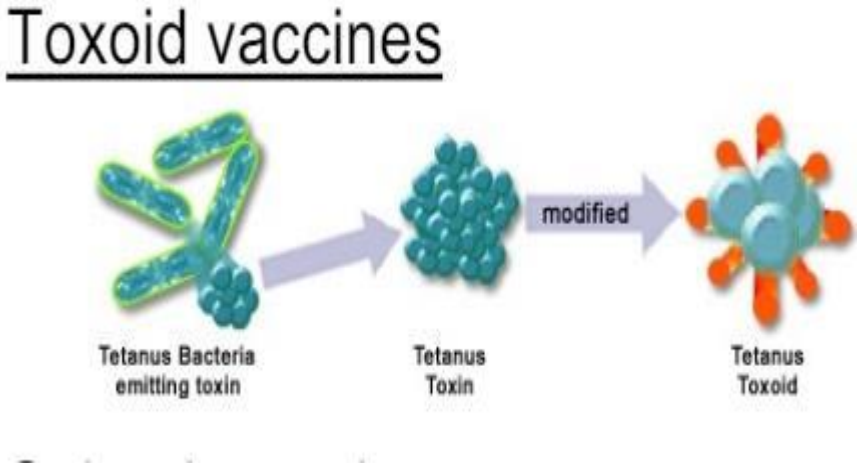
METHODS OF ATTENUATION:

- Passage through cell culture
- Mutagenesis (chemical or radiation-induced)
- Deletion of virulence genes
- Recombinant DNA technology

- **Advantages**:
- Induce strong immune response
- Mimic natural infection
- Provide long-term immunity
- **Disadvantages**:
- Risk of reversion to virulence
- May not be suitable for immunocompromised individuals
- Require careful handling and storage

TOXOID VACCINE:

- Toxoid vaccine is a type of vaccine that protects against diseases caused by bacterial toxins.
- Toxoid vaccines work by introducing a modified, harmless form of the toxin (called a toxoid) to the body, which triggers an immune response and produces antibodies that can recognize and neutralize the toxin.
- Examples of toxoid vaccines include:
- Diphtheria toxoid vaccine
- Tetanus toxoid vaccine
- Pertussis toxoid vaccine (whooping cough

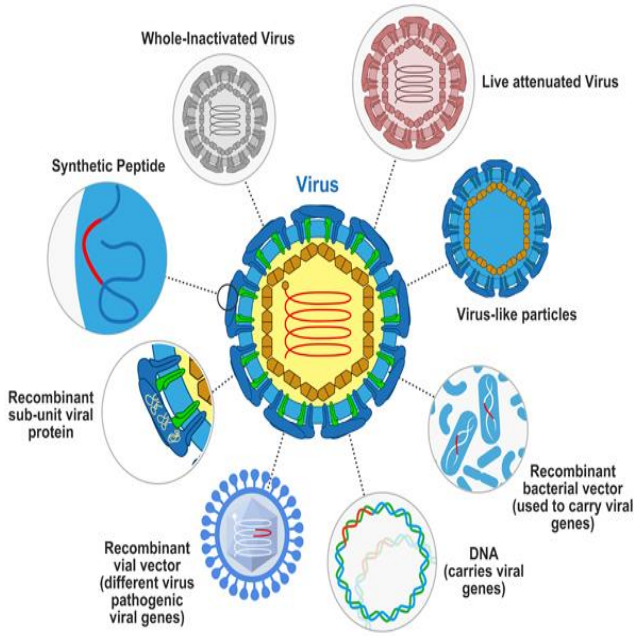


PRODUCTION:

- Isolating the toxin produced by the pathogen (bacterium). Purifying the toxin to remove other components.
- Inactivating the toxin using: Formaldehyde , Heat ,Radiation , Chemical modification.
- Converting the toxin into a toxoid (a harmless, modified form)
- Formulating the toxoid into a vaccine product.
- Testing for safety, potency, and purity.

RECOMBINANT VECTOR VAACINE:

- Recombinant vaccines are usually produced by benefiting from bacteria, yeast, mammalian, and insect cells.
- This type of vaccine requires the insertion and transference of the DNA section responsible for encoding the antigen.
- Among the mentioned cells, bacterial expression is the most frequently utilized type that does not need modifications associated with mammalians' and insects' cells .



PRODUCTION:

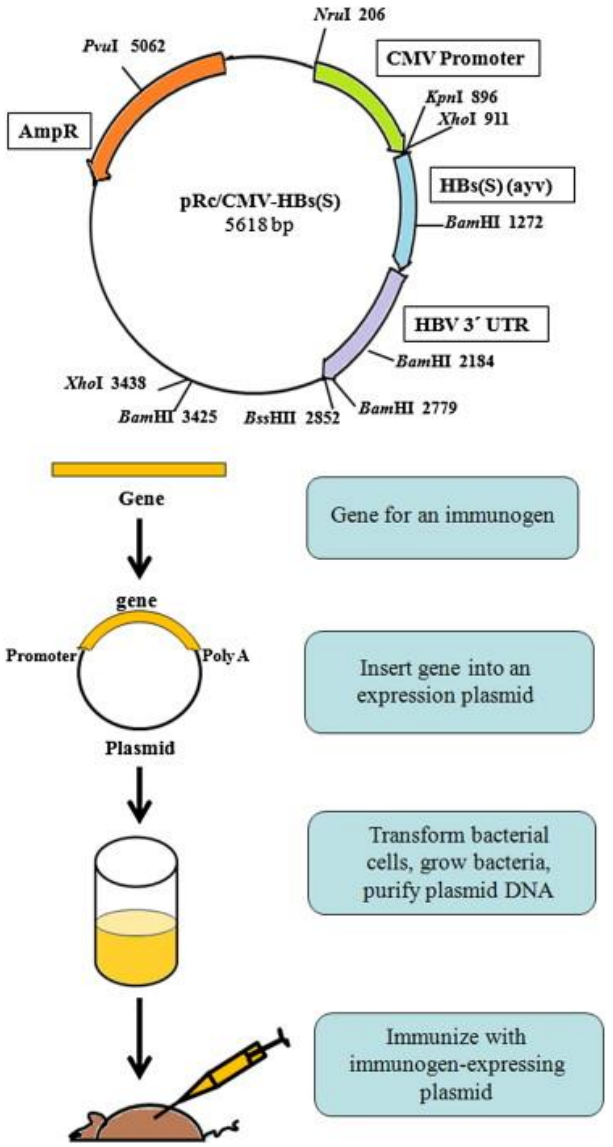
- Choosing a suitable vector (e.g., virus, bacterium, yeast) to deliver the antigen
- Cloning the gene encoding the desired antigen into the vector
- Expressing the antigen in the vector using recombinant DNA technology
- Purifying the vector-antigen complex
- Formulating the final vaccine product
- Testing for safety, potency, and purity

- **Types of recombinant vector vaccines**:
- Viral vectors (e.g., adenovirus, poxvirus, HIV)
- Bacterial vectors (e.g., Salmonella, E. Coli)
- Yeast vectors (e.g., Saccharomyces cerevisiae)
- **Advantages**:
- Flexibility in antigen design and expression
- Improved safety compared to traditional vaccines
- Enhanced immune response

DNA VACCINE:

- Naked DNA vaccines use plasmid DNA to encode and express a target protein within host cells.
- While DNA itself doesn’t trigger a strong immune response, the expressed protein can stimulate both humoral and cellular immunity.
- As of 2016, veterinary DNA vaccines exist (e.g., for West Nile Virus in horses), but human DNA vaccines are still in clinical trials for diseases like influenza and Ebola. Major safety concerns include potential DNA integration into the host genome, autoimmune reactions, and antibiotic resistance from plasmid production.

- The current evidence does not support these risks. DNA vaccines are advantageous because they are safe, stable, cost-effective, and easy to produce and store.
- Advances in delivery methods and protein expression optimization are ongoing, suggesting that human DNA vaccines may soon be available.



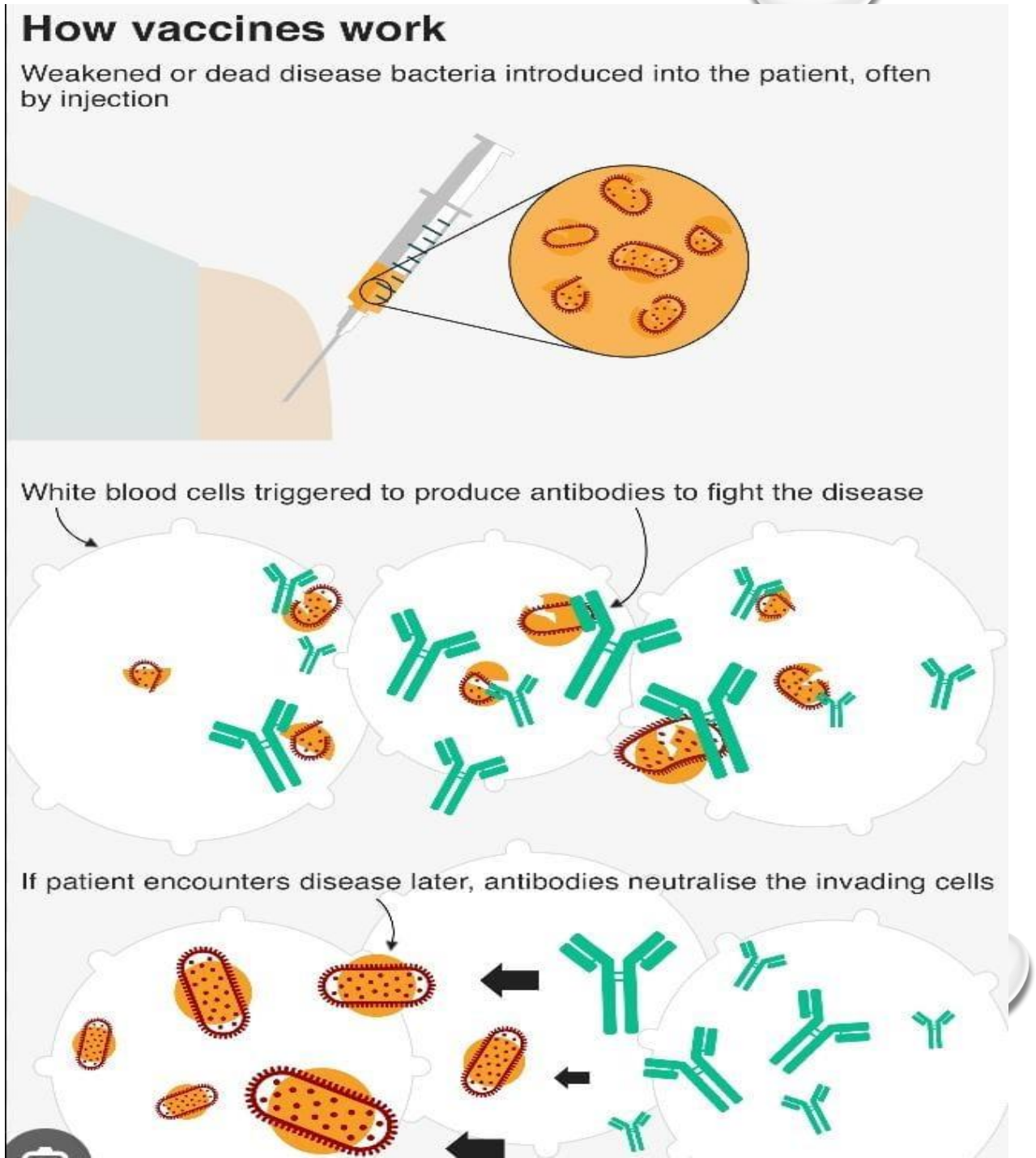
PRODUCTION:

- Designing the DNA plasmid with the desired antigen gene.
- Cloning the gene into the plasmid.
- Purifying the plasmid DNA.
- Formulating the DNA vaccine product (e.g., liquid, lyophilized).
- Testing for safety, potency, and purity.

- **Types of DNA vector vaccines:**
- Plasmid DNA vaccines
- Viral vector-based DNA vaccines (e.g., adenovirus, poxvirus)
- **Advantages**:
- Rapid development and production
- Flexibility in antigen design and expression
- Improved safety compared to traditional vaccines
- Stability and ease of storage

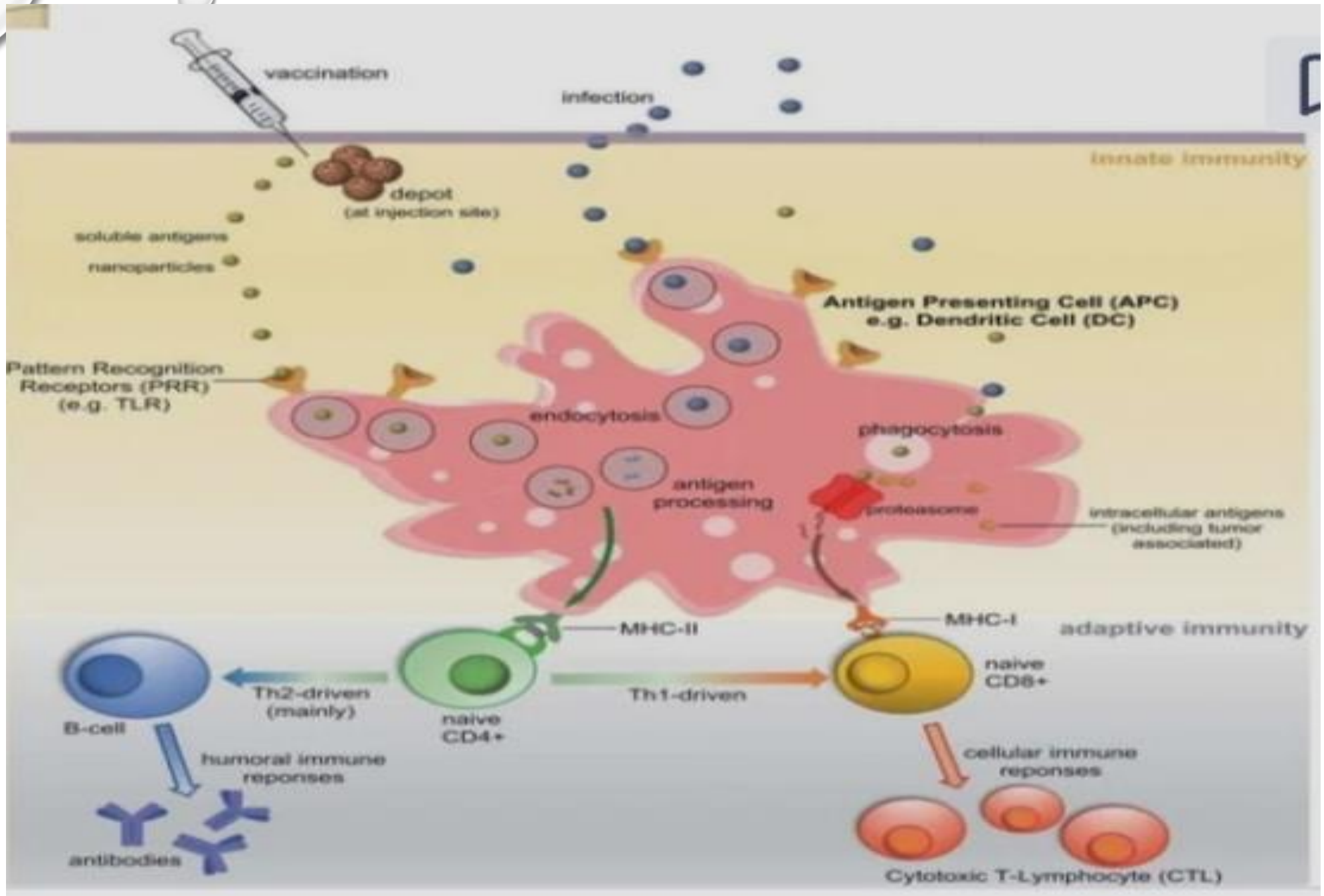
VACCINE:

- Vaccine is a biological preparation that stimulates the immune system to recognize and defend against specific pathogen promoting immunity and preventing infectious disease



SYNTHETIC PEPTIDE VACCINES:

- Synthetic peptide vaccine is a vaccine consisting mainly of 20 to 30 synthetic peptides.
- Synthetic peptide vaccine are usually considered to be safer than vaccines from microbial cultures.
- Creating vaccines synthetically has the ability to increase the speed of production. This is especially important in the event of a pandemic.
- The world's first synthetic vaccine was created in 1982 from diphtheria toxin by louis chedid and Michael sela .

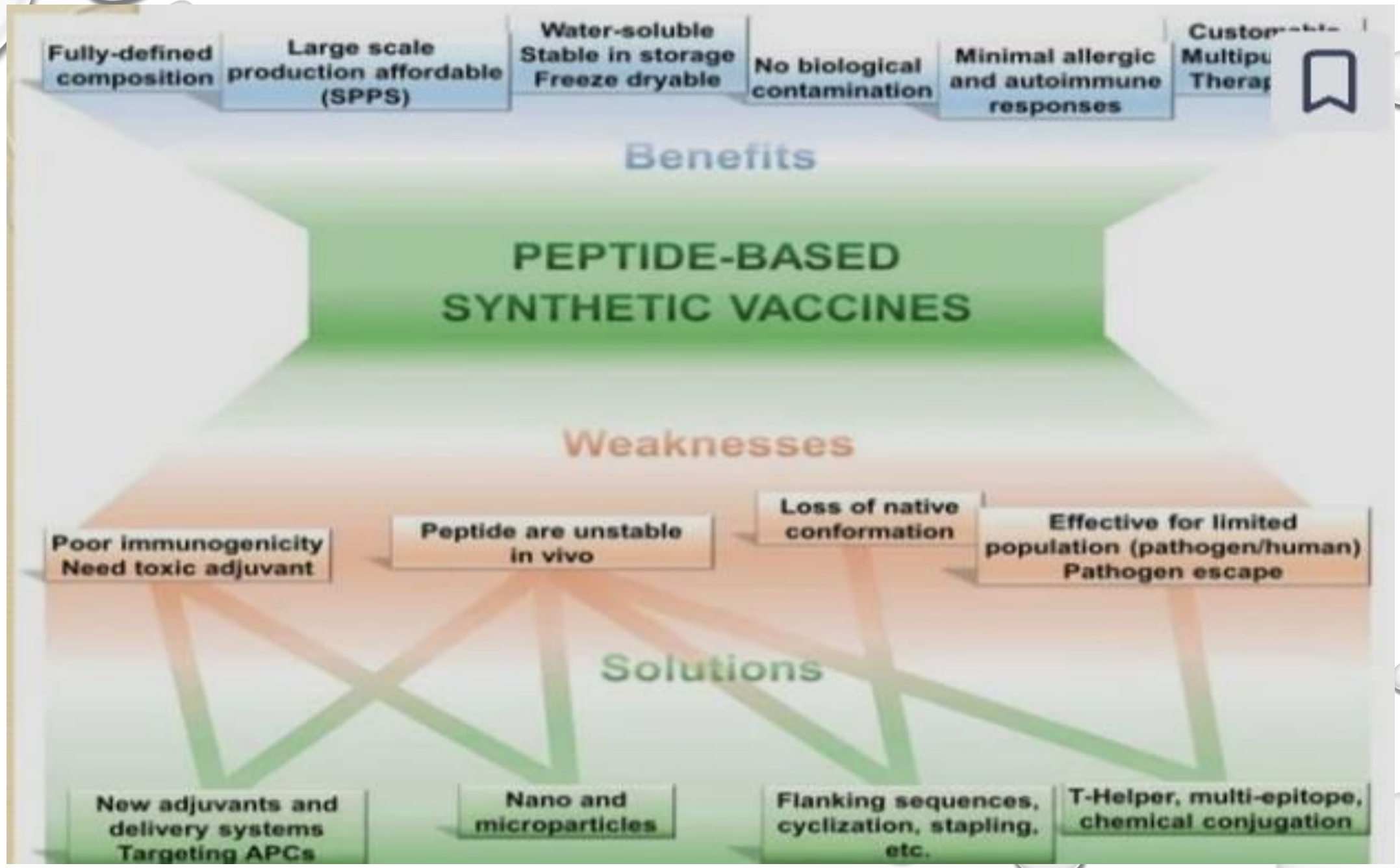

vaccination
infection
innate immunity
depot
(at injection site)
soluble antigens
nanoparticles
Antigen Presenting Cell (APC)
e.g. Dendritic Cell (DC)
Pattern Recognition
Receptors (PRR)
(e.g. TLR)
endocytosis
phagocytosis
antigen
processing
proteasome
intracellular antigens
(including tumor
associated)
MHC-II
MHC-I
adaptive immunity
naive
CD8+
Th2-driven
(mainly)
Th1-driven
B-cell
naive
CD4+
humoral immune
reponses
cellular immune
reponses
antibodies
Cytotoxic T-Lymphocyte (CTL)

ADVANTAGES:

- stable and relatively cheap to manufacture.
- Less quality assurance is required.
- Less toxic.
- Production and quality control is very simple.

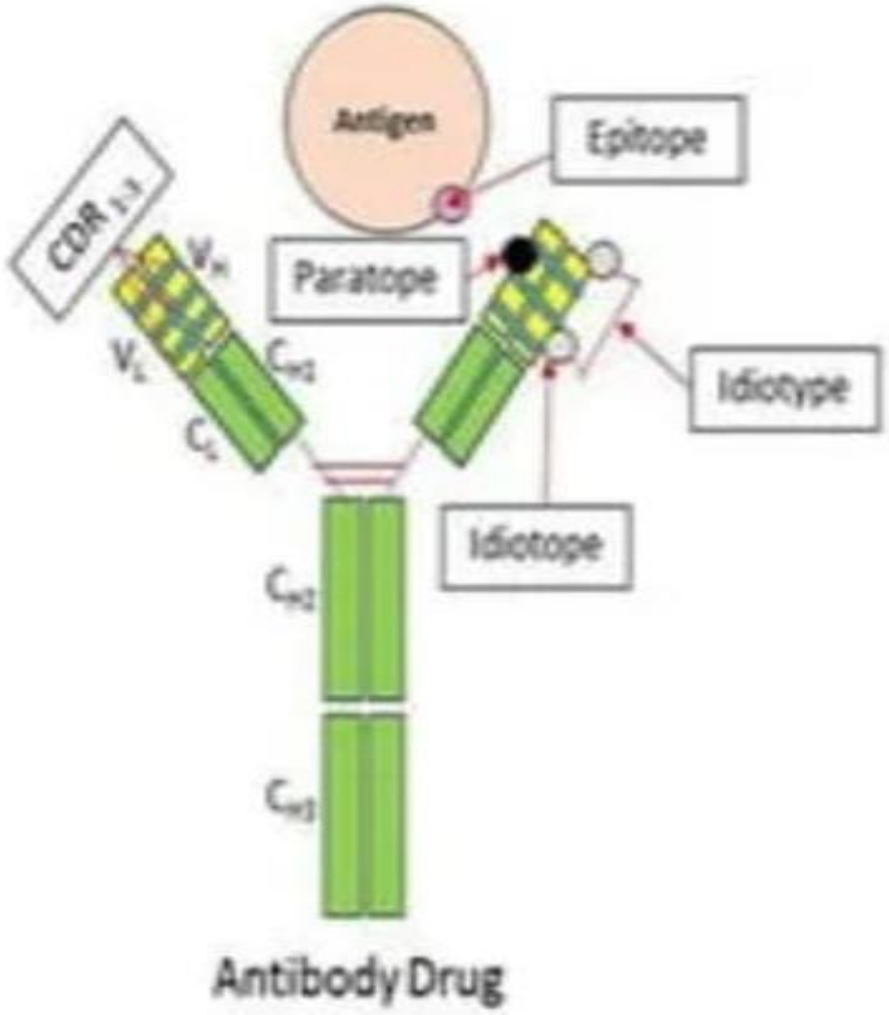
Disadvantage:

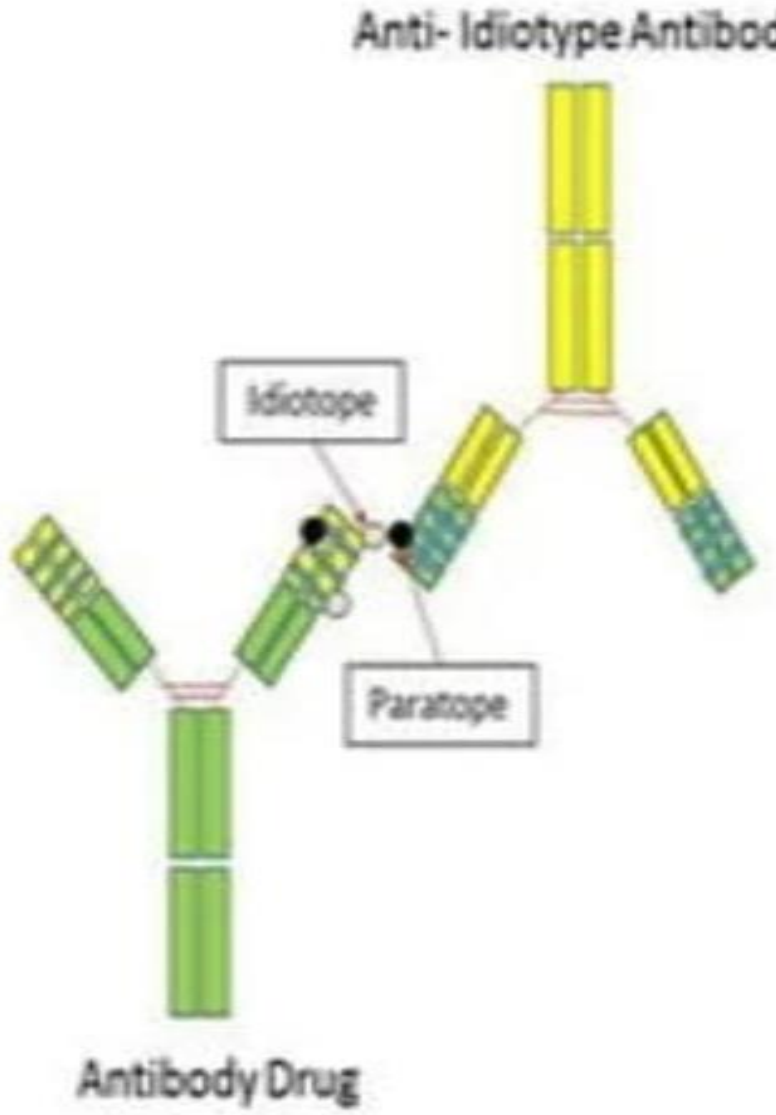
- They do not readily stimulate T cells.
- They are not applicable to all viruses.
- May be less immunogenic than conventional inactivated whole – virus vaccines.
- Requires adjuvant.
- Fails to elicit cell mediated immunity.


Fully-defined composition
Large scale production affordable (SPPS)
Water-soluble
Stable in storage
Freeze dryable
No biological contamination
Minimal allergic and autoimmune responses
Benefits
PEPTIDE-BASED SYNTHETIC VACCINES
Weaknesses
Poor immunogenicity
Need toxic adjuvant
Peptide are unstable in vivo
Loss of native conformation
Effective for limited population (pathogen/human)
Pathogen escape
Solutions
New adjuvants and delivery systems
Targeting APCs
Nano and microparticles
Flanking sequences, cyclization, stapling, etc.
T-Helper, multi-epitope, chemical conjugation

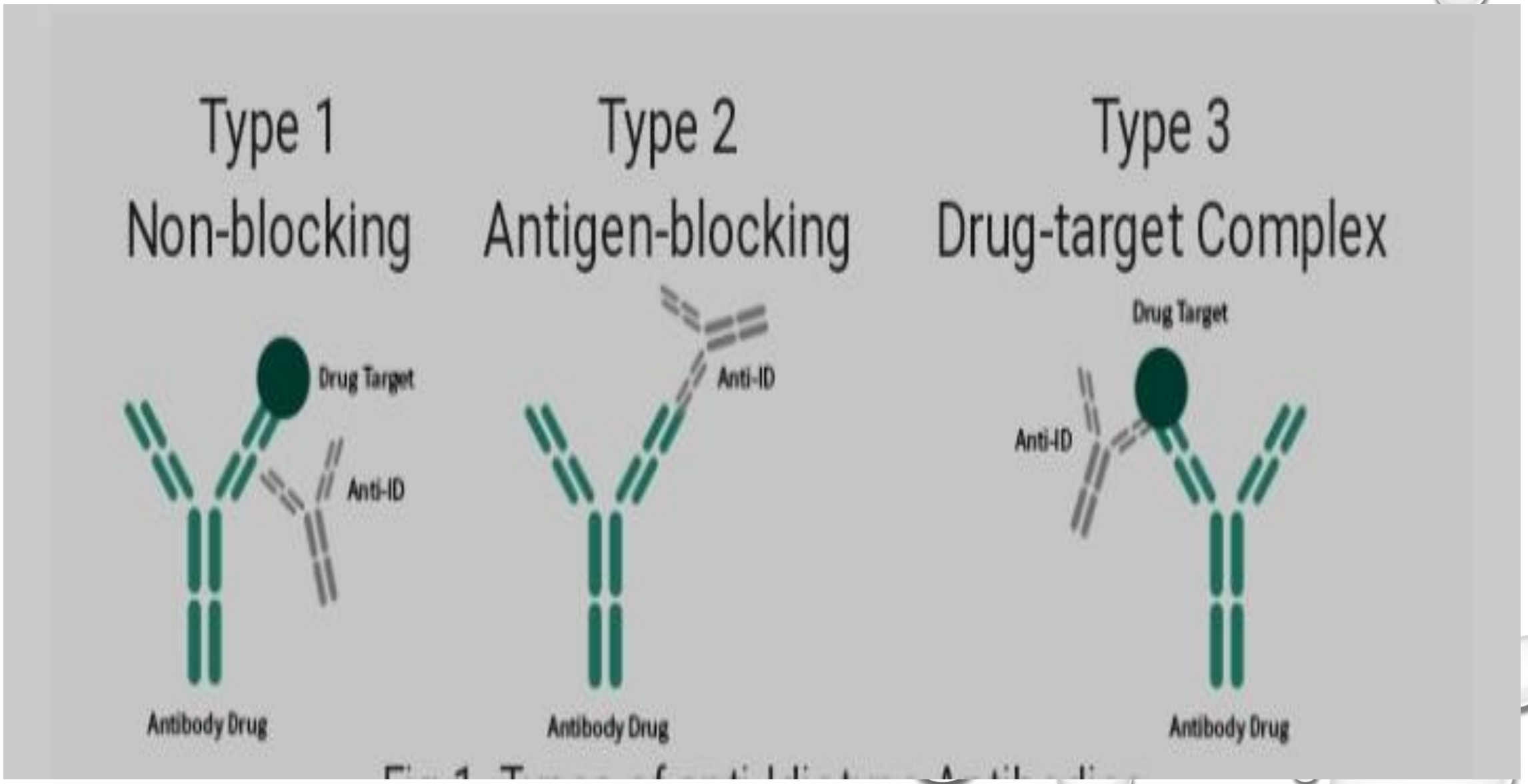
ANTI – IDIOTYPE VACCINE:

- Vaccine made of antibodies that see other antibodies as the antigen and bind to it.
- Anti-idiotype vaccines can stimulate the body to produce antibodies against tumor cells.
- Anti-idiotype antibody(Aid) is the anti-antibody aiming at group-specific antigen Epitope of the V region of the antibody molecule.
- Anti-idiotypic vaccines comprise antibodies that have 3D immunogenic regions, designated idiotopes, that consist of protein sequences that bind to cell receptors.
- Idiotopes are aggregated into idiotypes specific of their target antigen.


Antigen
Epitope
CDR 1-3
Paratope
Idiotype
Idiotope
Antibody Drug


Anti- Idiotype Antibody
Idiotope
Paratope
Antibody Drug

- Anti-idiotype antibody vaccine is a new type of immune biologics which was developed in the late 1970s.
- It developed towards practical areas and it had a big through in its production of vaccines, treatment of cancer and so on.
- Anti-idiotypes have many potential uses as viral vaccines, particularly when the antigen is difficult to grow or hazardous.
- They have been used to induce immunity against a wide range of viruses, including Hepatitis – B, Rabies, Newcastle disease virus and Reoviruses and Polioviruses.


Type 1
Non-blocking
Drug Target
Anti-ID
Antibody Drug
Type 2
Antigen-blocking
Anti-ID
Antibody Drug
Type 3
Drug-target Complex
Drug Target
Anti-ID
Antibody Drug

ADVANTAGES:

- Anti-idiotype antibodies can be used as positive controls in anti-drug antibodies (ADA) assays.
- They are also powerful tools to perform immunogenicity and PK/PD analysis during the process of therapeutic antibody development.
- Low toxicity: anti-idiotype vaccines are generally considered safe and well-tolerated, with minimal side effects.
- Flexibility: they can be designed to target a wide range of diseases, including cancer, infectious diseases, and autoimmune disorders

DISADVANTAGES:

- The production of ant-idiotype mAbs is challenging in syngeneic systems making anti-idiotype antibodies that mimic small ligands is challenging
- it is difficult and expensive to produce the anti-idiotype antibody with expression system.

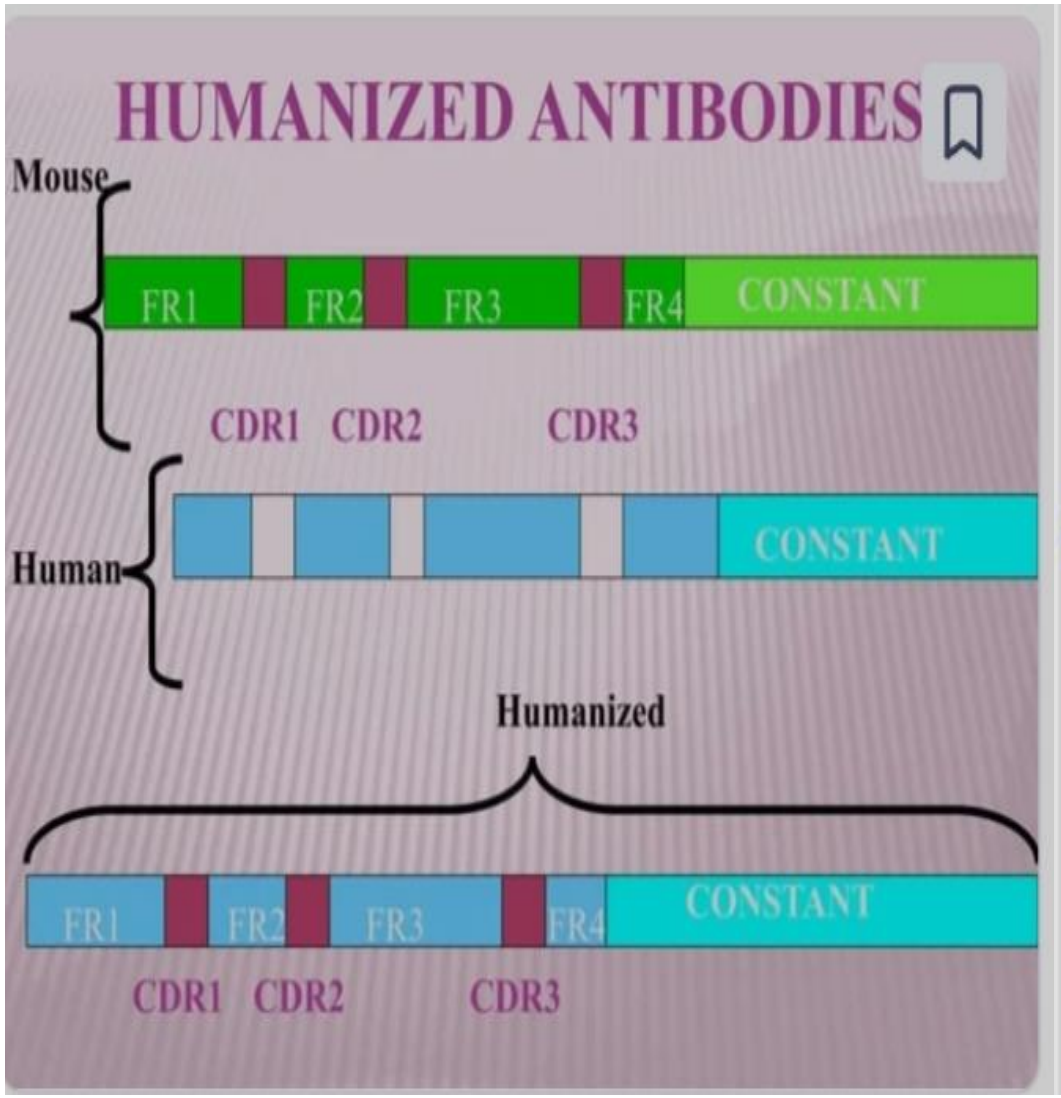
ANTIBODIES:

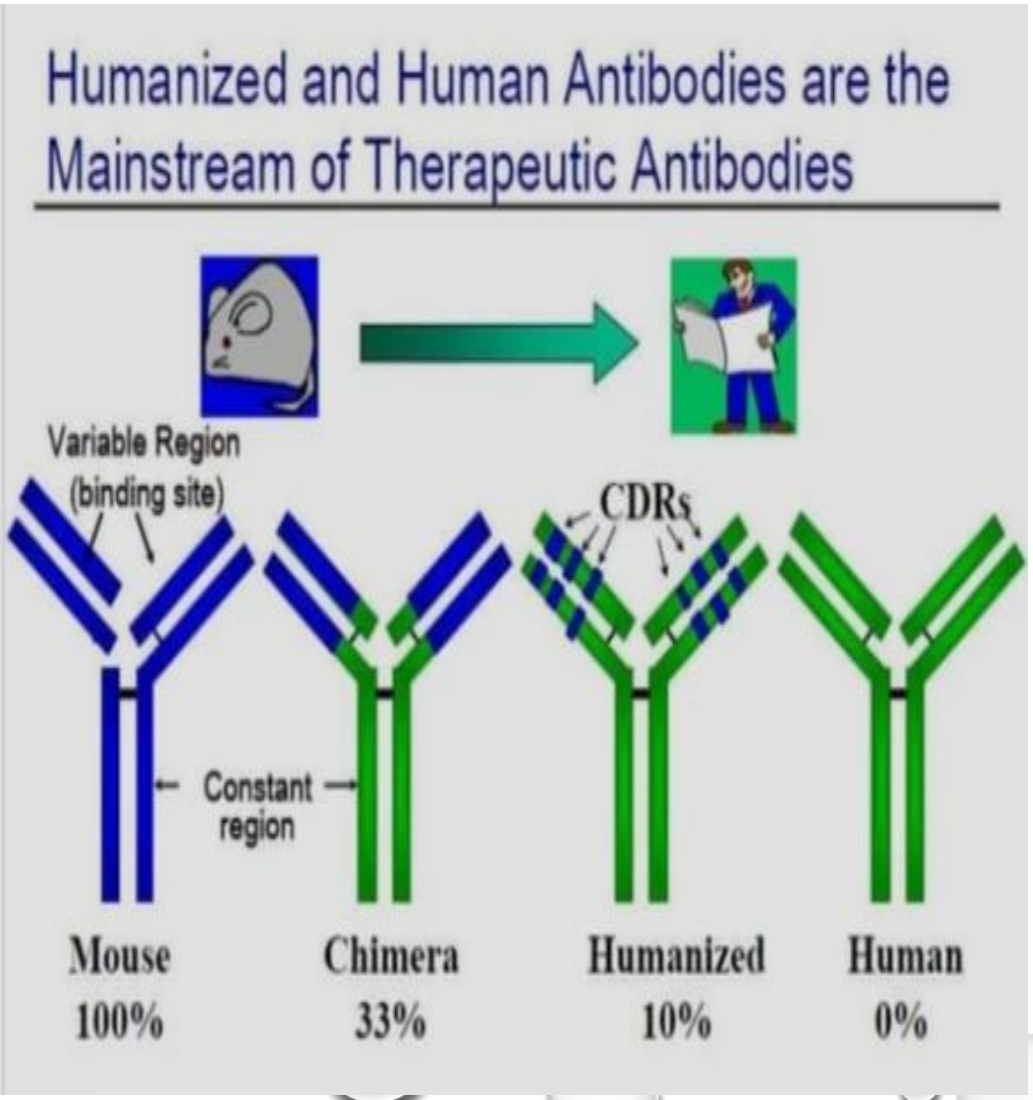
- Antibody is a protein used by the immune system to identify and neutralize foreign objects like bacteria and viruses.
- Each antibody recognizes a specific antigen unique to its target.
- Monoclonal antibodies are identical because they were produced by one type of immune cell.
- polyclonal antibodies are derived from different cell lines.

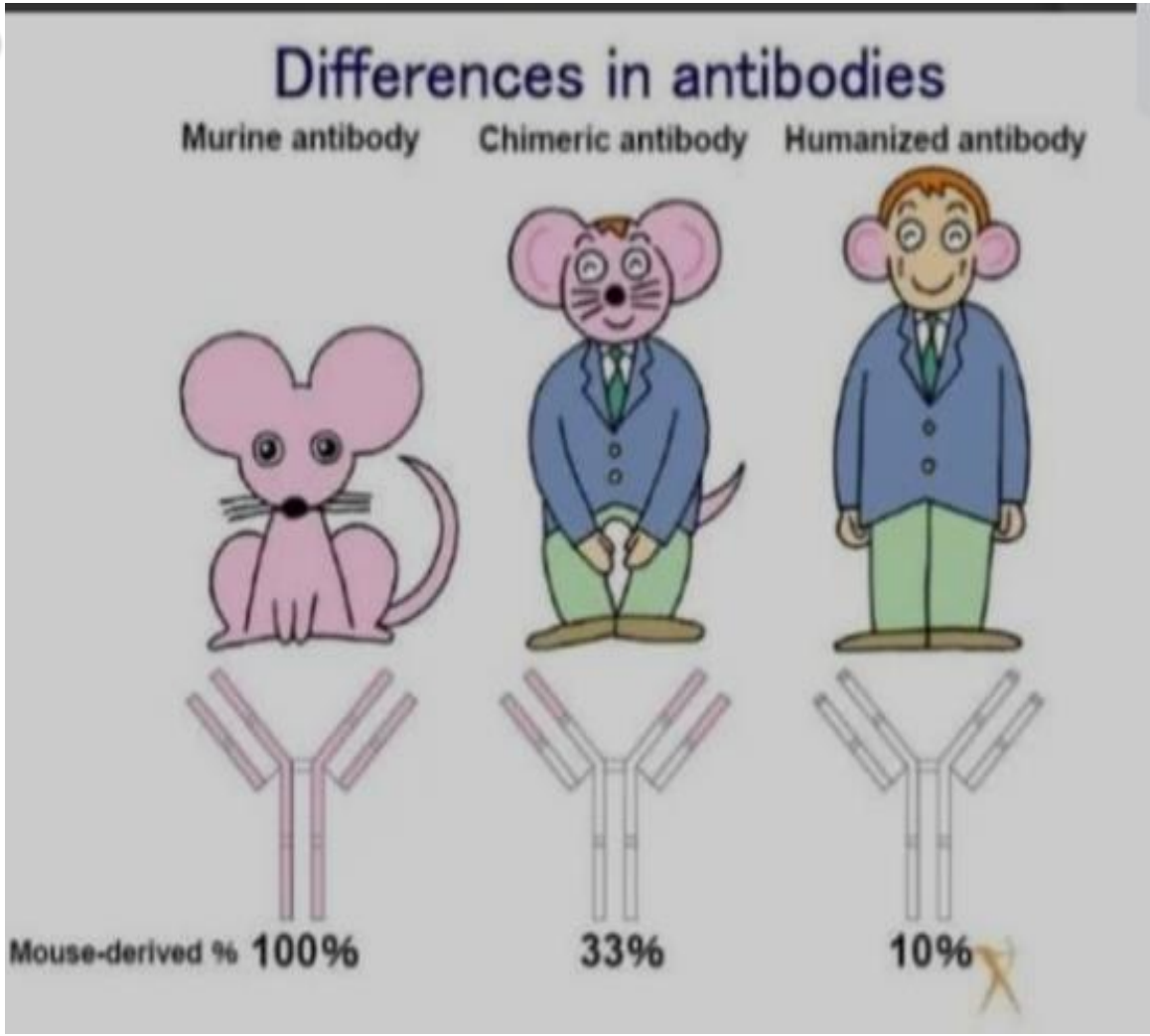
HUMANIZED ANTIBODY:

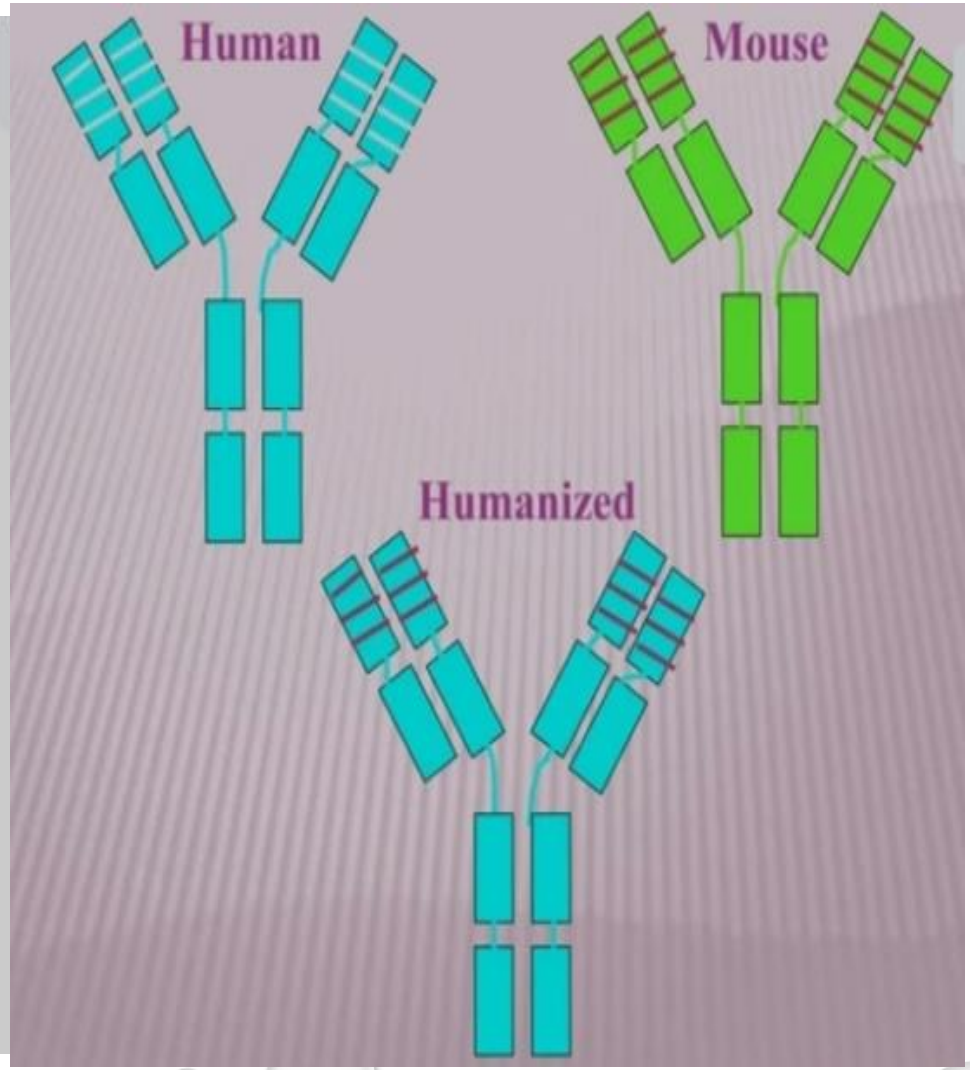
- Humanized antibodies are antibody from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.
- The process of “humanization” is usually applied to monoclonal antibodies developed for administration to humans (for eg antibodies developed as anti-cancer drugs).

- Ever since the discovery that monoclonal antibodies could be generated, scientists have targeted the creation of “fully” human products to reduce the side effects of humanised or chimeric antibodies.
- Two successful approaches have been identified: transgenic mice and phage display.
- As of November 2016, 13/19 cells “fully” human monoclonal antibody therapeutics on the market were derived from transgenic mice technology.
- These antibodies have been approved to treat cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis and viral infection.


HUMANIZED ANTIBODIES
Mouse
FR1
FR2
FR3
FR4
CONSTANT
CDR1
CDR2
CDR3
Human
CONSTANT
Humanized
FR1
FR2
FR3
FR4
CONSTANT
CDR1
CDR2
CDR3


Humanized and Human Antibodies are the Mainstream of Therapeutic Antibodies
Variable Region
(binding site)
CDRs
Constant
region
Mouse
100%
Chimera
33%
Humanized
10%
Human
0%


Differences in antibodies
Murine antibody
Chimeric antibody
Humanized antibody
Mouse-derived %
100%
33%
10%


Human
Mouse
Humanized

PLANTIBODIES:

- A plantibody is an antibody/proteins produced by genetically modified crops.
- Antibody are part of animal immune system and produce in plant by transforming them with antibody genes from animal.
- They are used as edible vaccines, diagnostic/therapeutic monoclonal antibodies, for disease resistance in plants.
- This was first done in 1989 with mouse antibody made by tobacco plant.
- Although plant do not naturally make antibody. Plantibody have shown the function in the same way as normal antibody.

TECHNIQUES :

- RIA(Radioimmunoassay)
- Elisa(enzyme linked immune sorbent assay)
- Immunofluorence southern blot analysis

ADVANTAGES:

- Large scale production of plantibodies for immunotherapy.
- Beneficial to human , plants as well as economy.
- No risk of transmitting diseases to human.
- Eliminate the toxic compound.

Applications:

- Antibodies generated by plants are cheaper , easier to manage and safer to use than those obtained from animals.
- Treatment of illness such as immune disorder, cancer and inflammatory disease.
- Plantibodies are close to passing clinical trail.

PRODUCTION OF POLYCLONAL AND MONOCLONAL ANTIBODIES

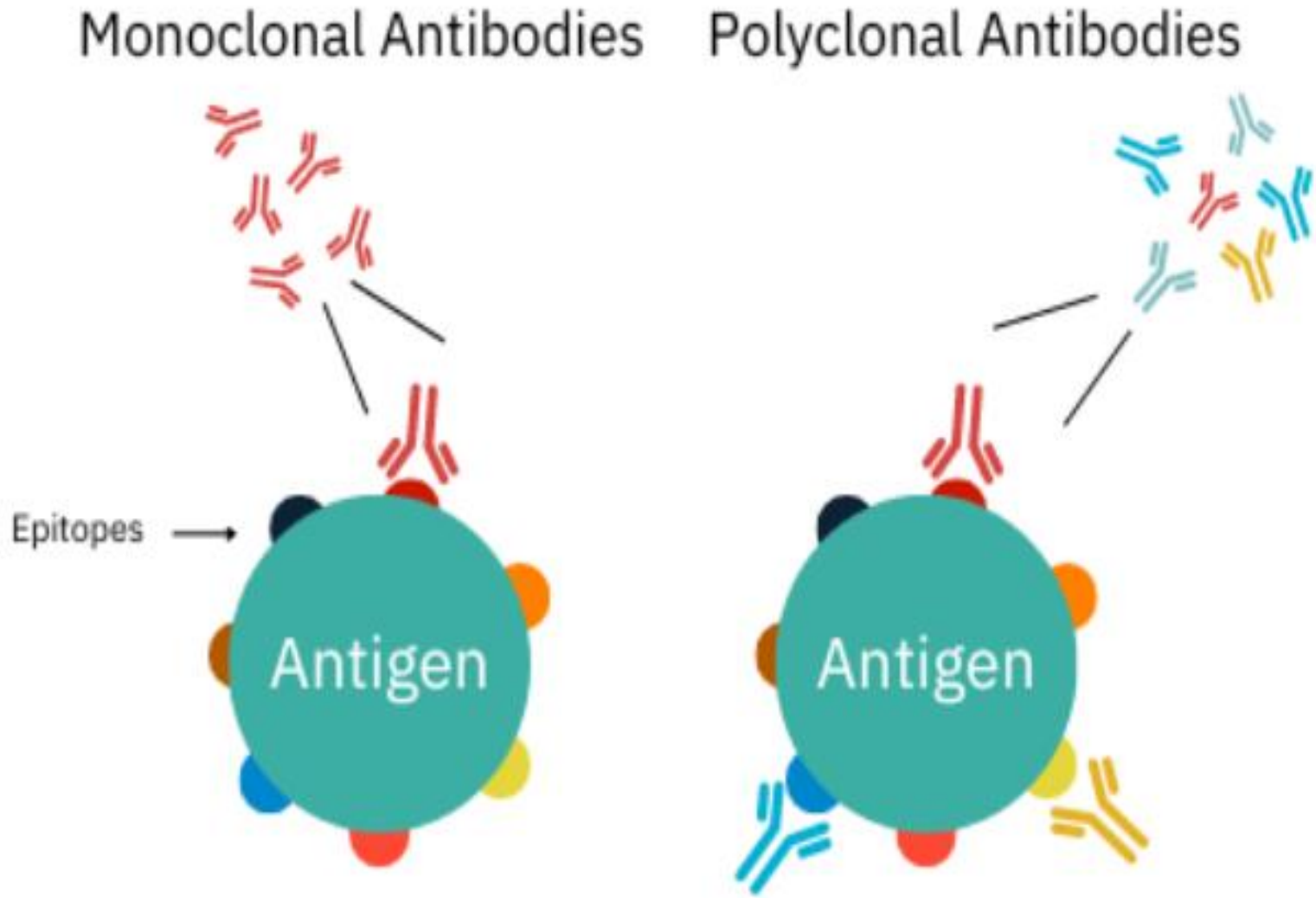
ANTIBODIES

Antibodies are proteins produced by the immune system that recognize and bind to specific foreign substances called **antigens**, such as bacteria, viruses, and toxins, in order to neutralize or eliminate them from the body.

- They are Y-shaped proteins that belong to the immunoglobulin superfamily.
- Antibodies can neutralize antigens directly or tag them for attack by other parts of the immune system
- They are produced by specialized **white blood cells called B cells** when stimulated by an antigen
- Antibodies remain in the body for months after an infection, providing extended immunity
- There are five main classes of antibodies (IgA, IgD, IgE, IgG, IgM) that differ in their location and function

MONOCLONAL ANTIBODIES -principle

- Monoclonal antibodies (mAbs) are antibodies that are identical and produced from a single clone of B cells, which are derived from a unique parent cell.
- The principle behind monoclonal antibodies is their ability to bind specifically to a single epitope on an antigen, allowing for precise targeting in various applications, including diagnostics and therapeutics.
- The term "monoclonal" signifies that these antibodies originate from one type of immune cell, ensuring uniformity in their structure and function. This contrasts with polyclonal antibodies, which are derived from multiple B cell lineages and can recognize different epitopes on the same or different antigens.


Monoclonal Antibodies
Polyclonal Antibodies
Epitopes
Antigen
Antigen

TECHNIQUES

- Monoclonal antibodies are produced by immunizing (injection an immunogen) an animal, often a mouse, multiple times with a specific antigen.
- After the immunogen has caused an immune response, the B-cells from the spleen are removed.
- Since normal B cells are unable to proliferate forever, they are fused with immortal, cancerous B cells called myeloma cells, to yield hybridoma cells.

- All of the cells are then placed in a selective medium (Hypoxanthine Aminopterin Thymidine -HAT) that allows only the hybridomas to grow; unfused myeloma cells cannot grow, and any unfused B cells die off.
- Mechanism of grow hybridoma cell in this selective media:

B cell is contain a gene hypoxanthine-guanine phosphoribosyltransferase (HGPRT) but myeloma don't have it this gene.

- This gene is necessary for replication of the cell.
- This HAT media only cell that have HGPRT positive will survive ,while cells HGPRT negative will die. So, HAT media only kill HGPRT negative cells only.

- The hybridomas, which are capable of growing continuously in culture while producing antibodies, are then screened for the desired mAb.
- Those producing the desired mAbs are grown in tissue culture; the culture medium is harvested periodically and mAbs are purified from the medium.
- Once a monoclonal antibody hybridoma clone is created, it is a stable renewable source of antibodies.

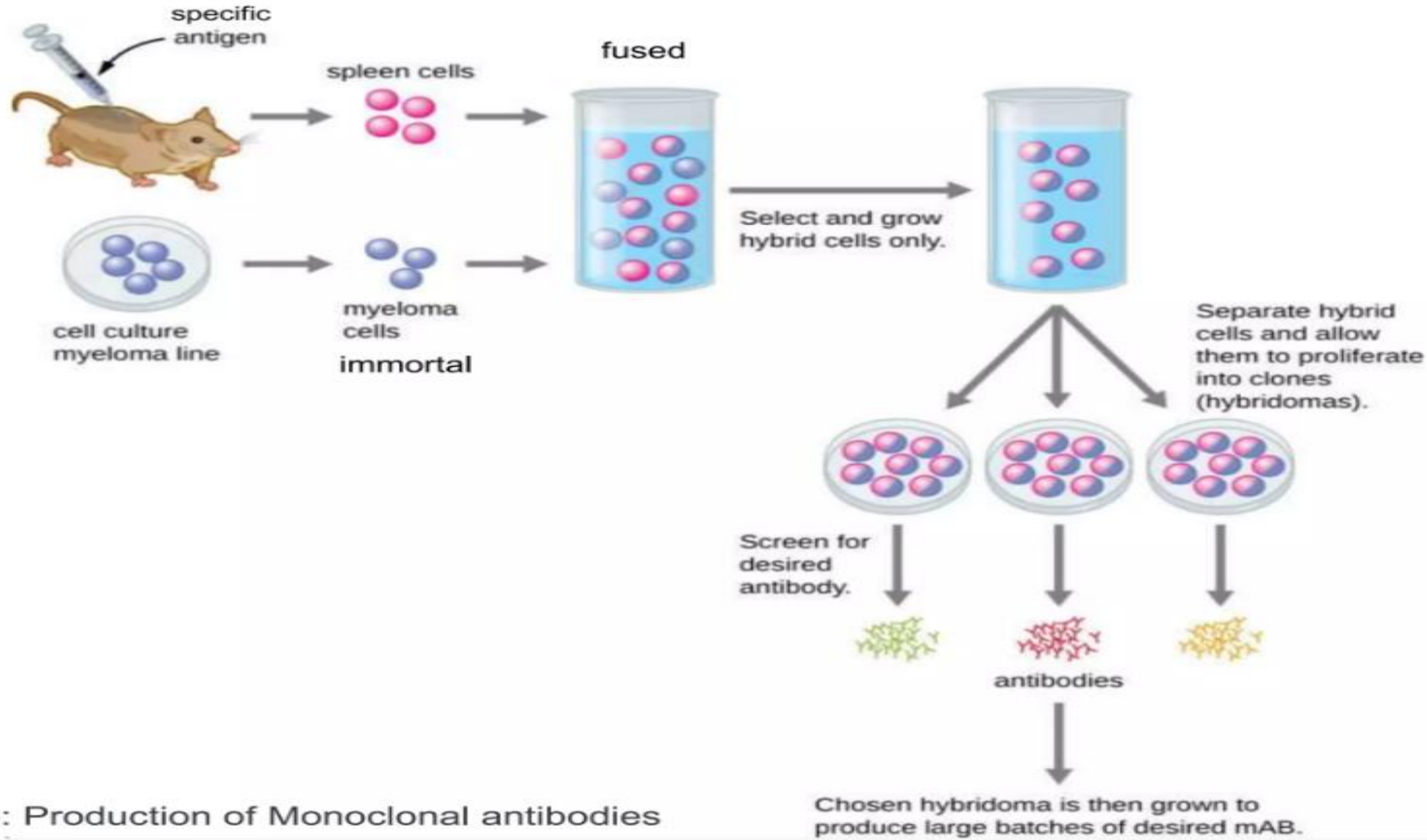


Figure: Production of Monoclonal antibodies

Hybridoma protocol

Immunization

Spleen

Propagation of mouse myeloma cells

Mouse challenged with antigen

Myeloma cell

Can propagate in culture for long time

Can not produce specific antibodies

Testing antibody response by ELISA

Spleen cells (containing B cells)

Produce specific antibodies

Can not propagate in culture for long time

Unfused B cells

Fused cells

Fused myeloma with spleen cell (B cell) propagate

Unfused myeloma cells

Fused myeloma cell

Cell fusion by PEG/DMSO

Die

Die

Die

Die

Propagation of positive hybrid clones in culture

Propagation of positive hybrid clones in mice

Propagation of cells in selected media (HAT)

Propagation in HT media

Screening of positive secreting clone by ELISA

Isotyping of generated antibodies

APPLICATION

- **Therapeutic Applications-** Cancer treatment: Monoclonal antibodies can target specific antigens on cancer cells, leading to their destruction. Examples include rituximab (for non-Hodgkin's lymphoma) and trastuzumab (for HER2-positive breast cancer).
- **Autoimmune diseases**: Monoclonal antibodies help in treating diseases like **rheumatoid arthritis** (e.g.,infliximab for TNF-α inhibition). -
- **COVID-19:** Monoclonal antibodies such as casirivimab and imdevimab have been used for treating or preventing COVID-19.
- **Research Applications**-Protein detection: mAbs are used in Western blotting, immunohistochemistry (IHC)to detect specific proteins in research and clinical samples.
- **Cell sorting**: Monoclonal antibodies are used in techniques like fluorescence-activated cell sorting (FACS) to isolate specific cell populations.

ADVANTAGES

- Can produce large quantities of identical antibodies.
- Have **high specificity** to a single epitope which reduces the risks for cross reactivity.
- Better results in assays requiring quantification of the protein levels
- Very efficient in affinity purification.

DISADVANTAGES

- More expensive.
- More sensitive to pH and buffer conditions.

POLYCLONAL ANTIBODIES PRINCIPLE

- Polyclonal antibodies (pAbs) are produced by multiple B cell clones, each targeting **different epitopes** on the same antigen.
- This results in a mixture of antibodies with varied specificities.
- The principle involves immunizing an animal with an antigen, triggering a diverse immune response, followed by collecting and purifying the resulting antibodies for broad and sensitive antigen recognition.

TECHNIQUES

- A collection of antibodies which are grown from **different B cells**. This makes them capable of recognizing multiple epitopes on the same antigen.
- Antibodies used for research and diagnostic purposes are often obtained by **injecting a lab animal** such as a rabbit with a specific antigen.
- Within a few weeks, the animal's immune system will produce high levels of antibodies specific for the antigen.

- These antibodies can be harvested in an **antiserum,** which is whole serum collected from an animal following exposure to an antigen.
- Antiserum obtained from animals will not only contain antibodies against the antigen artificially introduced in the laboratory, but it will also contain antibodies to any other antigens to which the animal has been exposed during its lifetime.
- For this reason, **antisera** must first be "purified" to remove other antibodies before using the antibodies for research or diagnostic assays.

specific

1 Inject antigen into rabbit.

2 Antigen activates B cells.

3 Plasma B cells produce polyclonal antibodies.

4 Obtain antiserum from rabbit containing polyclonal antibodies.

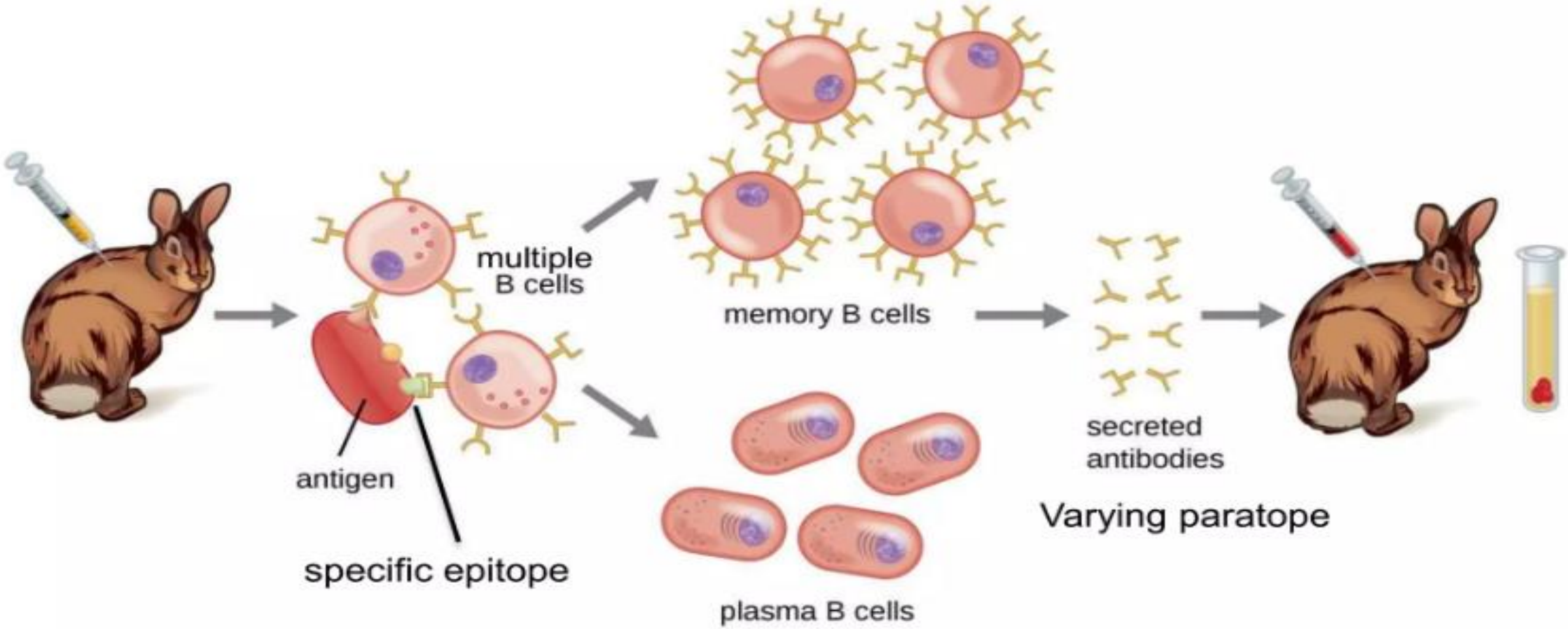


Figure: This diagram illustrates the process for harvesting polyclonal antibodies produced in response to an antigen.

APPLICATION

- **Anti-venom therapy**: Polyclonal antibodies are commonly used in anti-venom treatments for snake bites, scorpion stings, and other venomous animals. They neutralize a broad range of venom components.
- **Immune globulin therapy**: Polyclonal antibodies derived from human plasma (e.g., intravenous immunoglobulin, IVIG) are used to treat immune deficiencies, autoimmune diseases, and some viral infections (e.g., rabies, hepatitis B).
- **Infectious diseases**: Polyclonal antibodies are used to treat infections by neutralizing pathogens or their toxins, like tetanus antitoxin.

ADVANTAGES

- Inexpensive
- Easy to store
- Quick to produce
- Ready to use in under four months.
- High affinity as the antibodies bind to more than one epitope, they can help amplify the signal from target protein even with low expression level.

DISADVANTAGES

- Higher potential for cross reactivity.
- The affinity purification of the serum will typically be required to minimize cross reactivity.

Reference

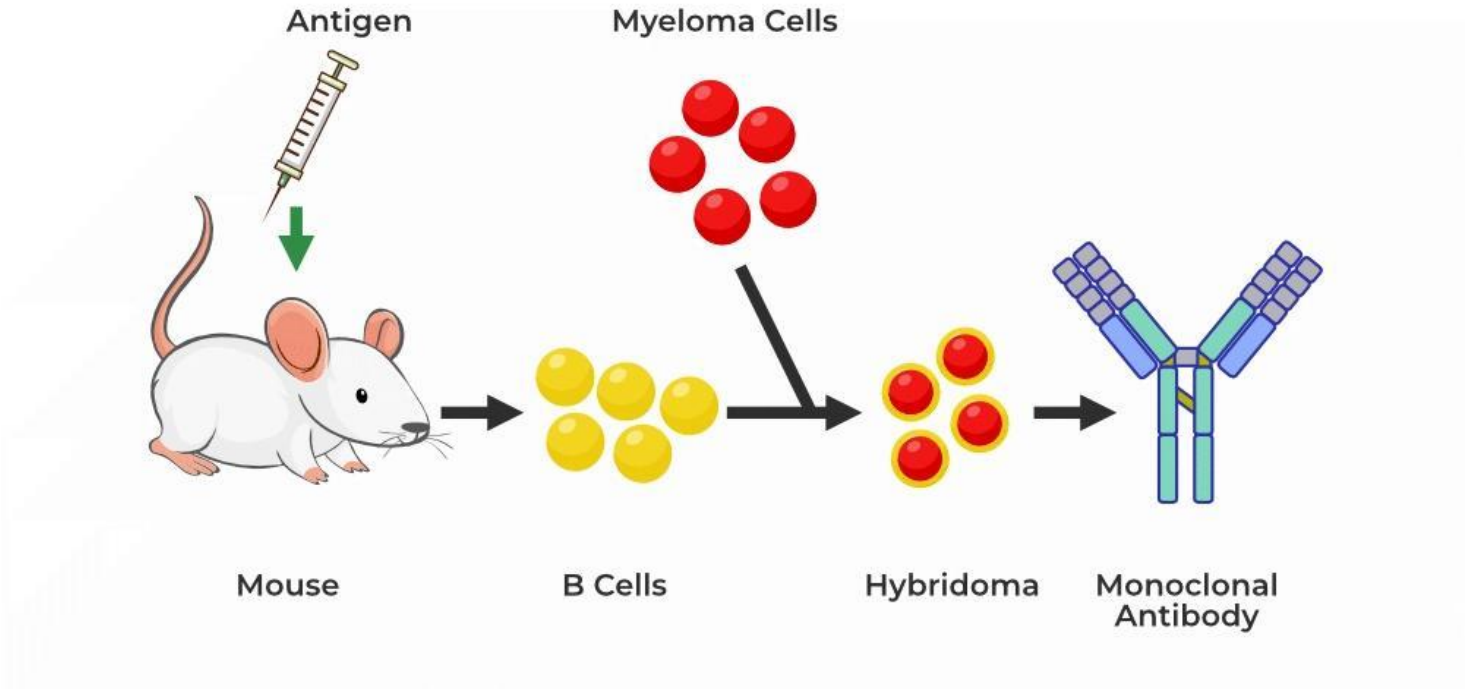
- https://www.biosynth.com/blog/monoclonal-and-polyclonal-antibody-applications
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4598607/

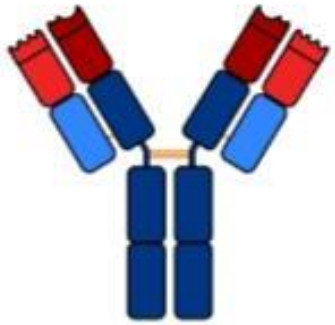
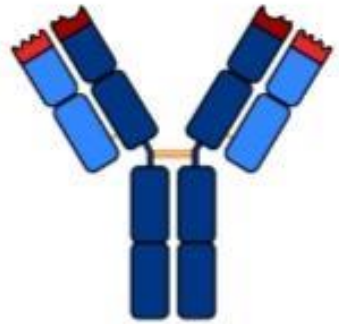
GENETICALLY ENGINEERED ANTIBODIES

- **Genetic engineering antibodies are novel recombinant antibody molecules with improved antigen specificities and effector functions,**
- **which produced by the recombinant DNA and protein engineering technologies.**

GENETICALLY ENGINEERED ANTIBIOTICS TYPES

- CHIMERIC ANTIBODIES
- HUMANIZED ANTIBODIES
- FULLY HUMAN ANTIBIOTICS
- BISPECIFIC ANTIBODIES



	Mouse monoclonal	Chimeric monoclonal	Humanized/ CDR-grafted monoclonal	Phage display synthetic monoclonal	Fully human/ transgenic human monoclonal
					
e.g.	Muromonab ("Muromomab") (Orthoclone OKT3)	Rituximab (Rituxan)	Bevacizumab (Avastin)	Adalimumab (Humira)	Panitumumab (Vectibix)
	1975 Köhler & Milstein	1984 Morrison et al.	1986 Jones et al.	1990 McCafferty et al.	1994 Lonberg et al. & Green et al.

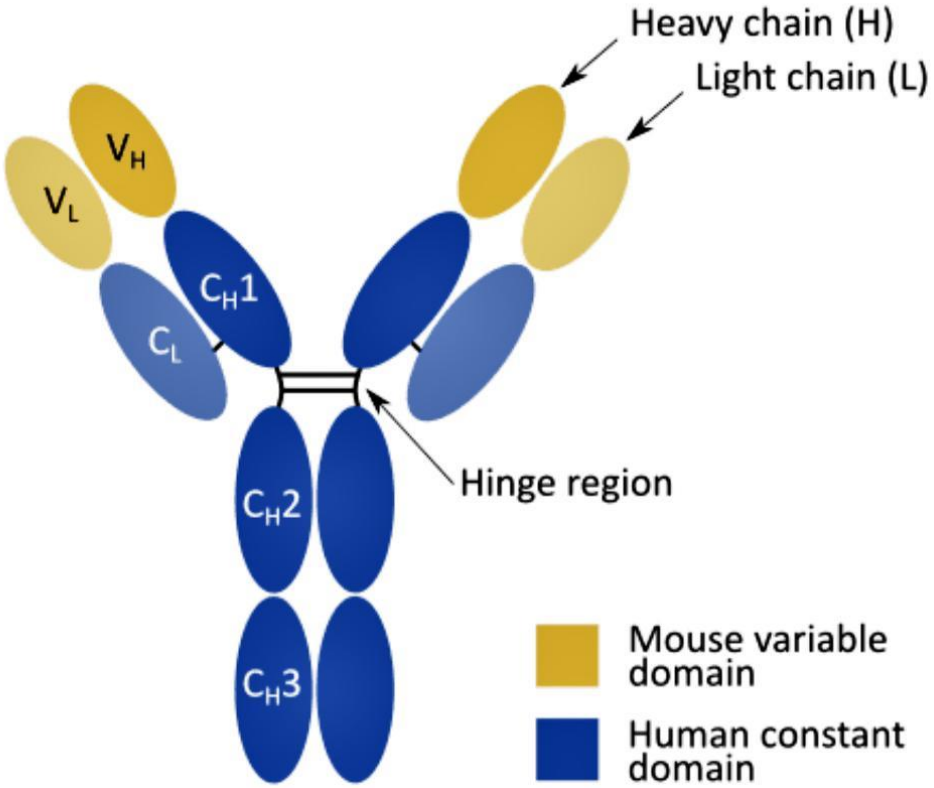
Decreasing likelyhood of immune reaction towards the antibody

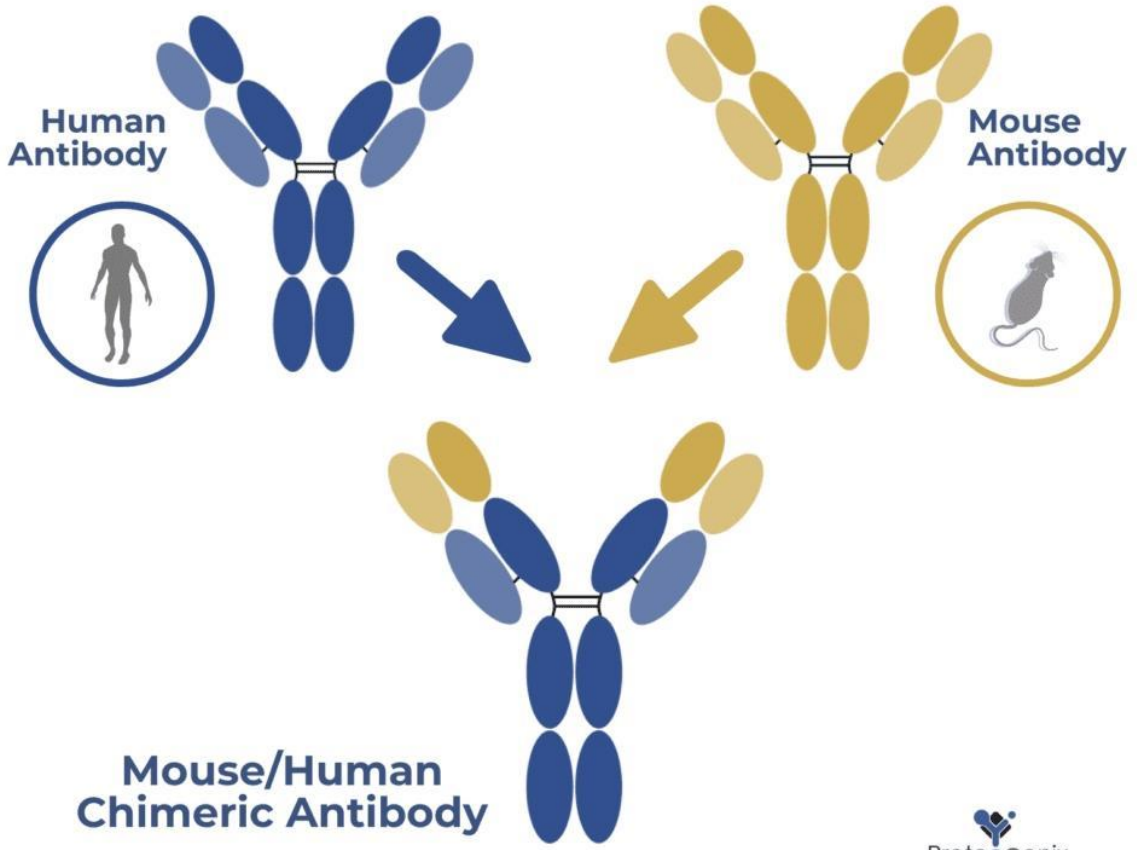
GENETICALLY ENGINEERED ANTIBODIES TYPES

Chimeric Antibodies:

- **Combine the variable region (antigen-binding part) of a mouse antibody with the constant region of a human antibody.**
- **Reduces immune responses against mouse components when used in humans.**
- **Example: Rituximab (used in lymphoma treatment).**

What are chimeric antibodies

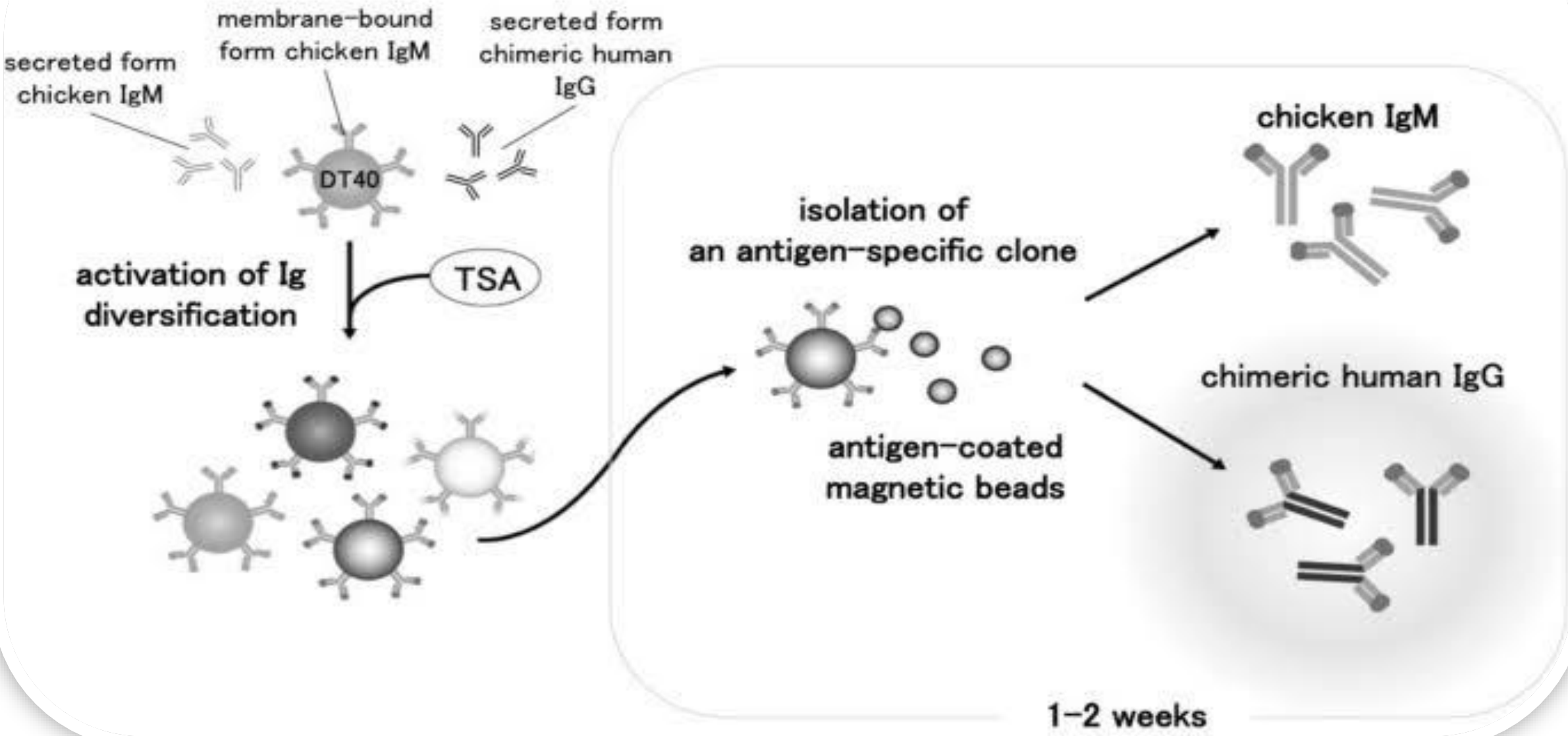




ProteoGenix
Accelarating your way to the clinic

Autonomously Diversifying Library System

combined with the use of a chimeric IgG producing cell-line



First, the spleen containing B cells from an immunized mouse is collected. The B cells are fused with myeloma cells to construct hybridoma and isolate a clone producing antigen-specific IgG. The DNA sequences coding mouse VH and VL are then isolated from the clone, as well as the DNA sequences coding human immunoglobulin constant regions from human cells. Mouse/human chimeric genes are constructed by genetic engineering and transfected into mammalian cells. Finally, the clone revealing a high level expression of chimeric IgG is selected and the IgGs are purified from the culture supernatant.

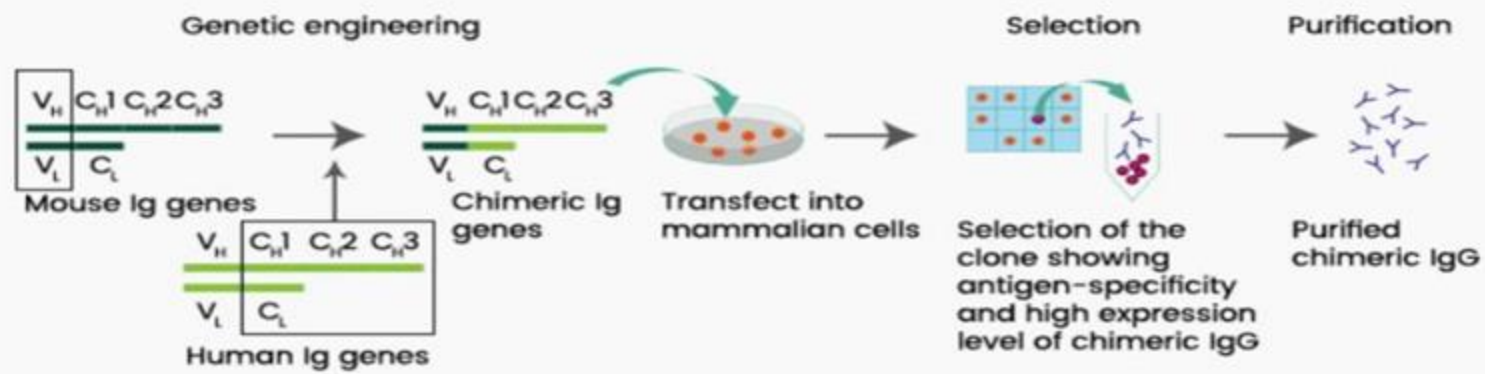
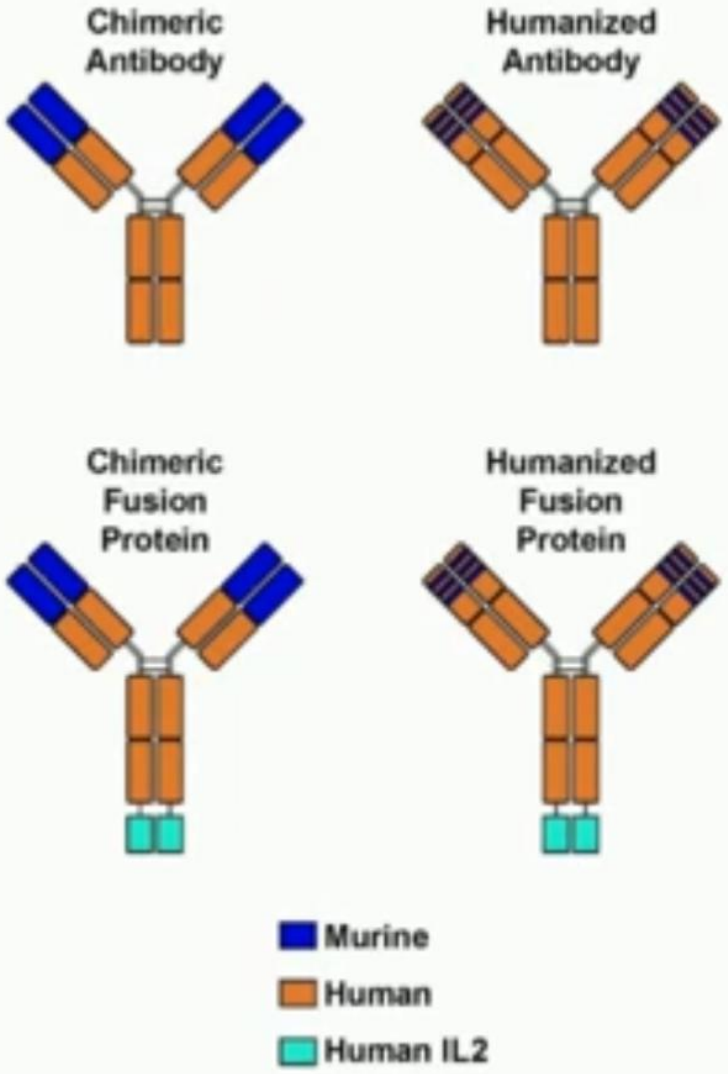


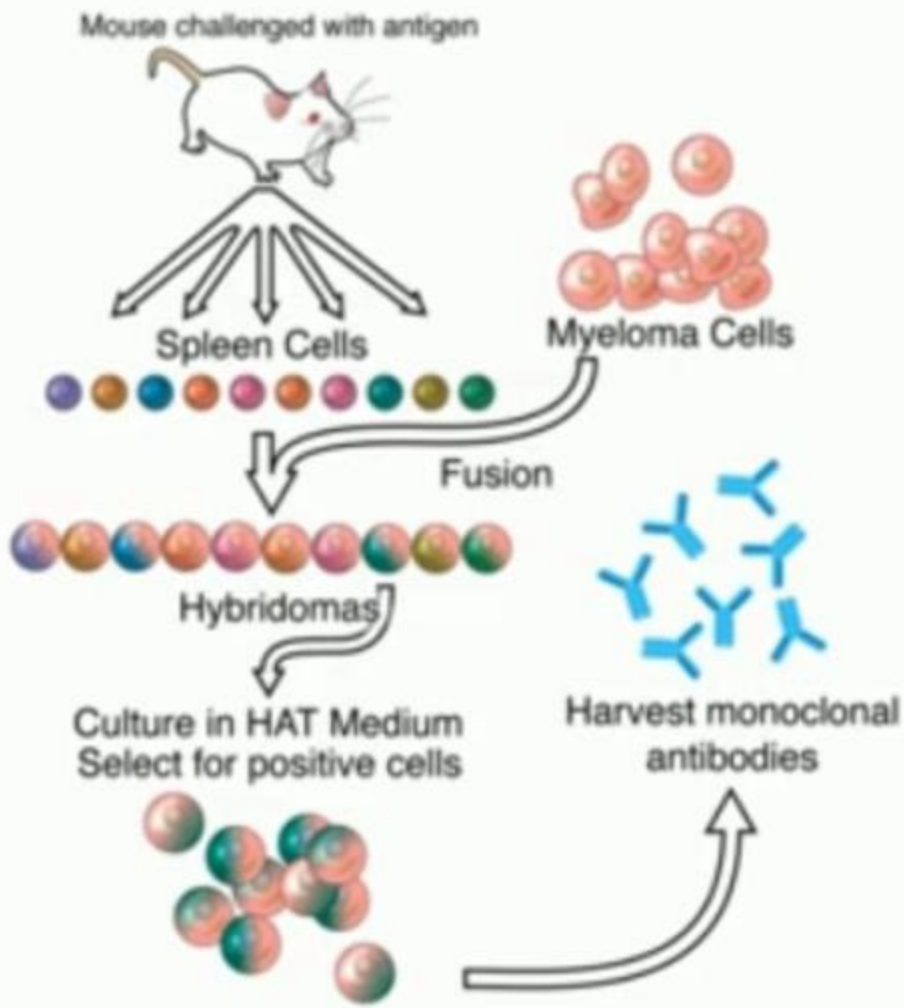
Fig 1. How to generate human-mouse chimeric monoclonal antibodies

Humanized Antibodies:

- Only the antigen-binding (complementarity-determining regions or CDRs) part of the mouse antibody is retained, while the rest of the structure is human.
- Significantly reduces immunogenicity compared to chimeric antibodies.
- Example: Trastuzumab (Herceptin), used in breast cancer.



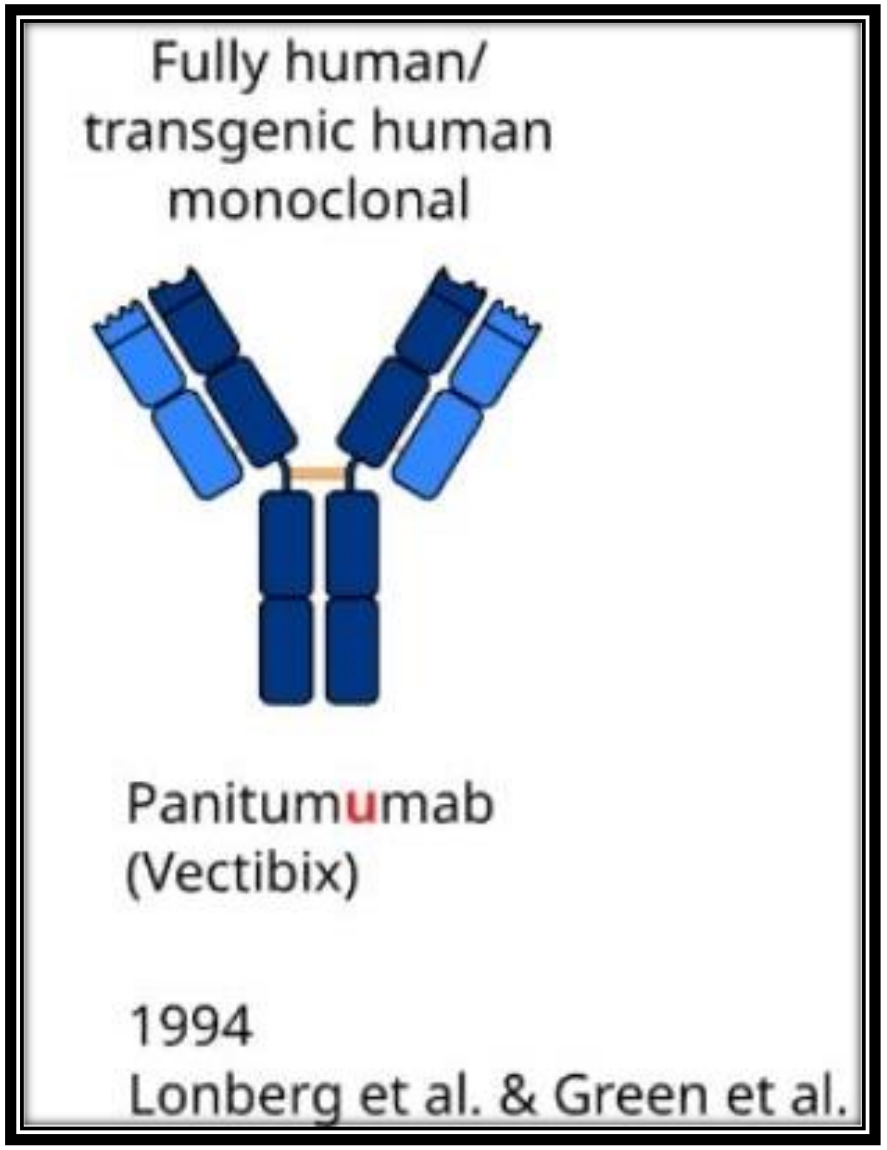
An antibody from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.

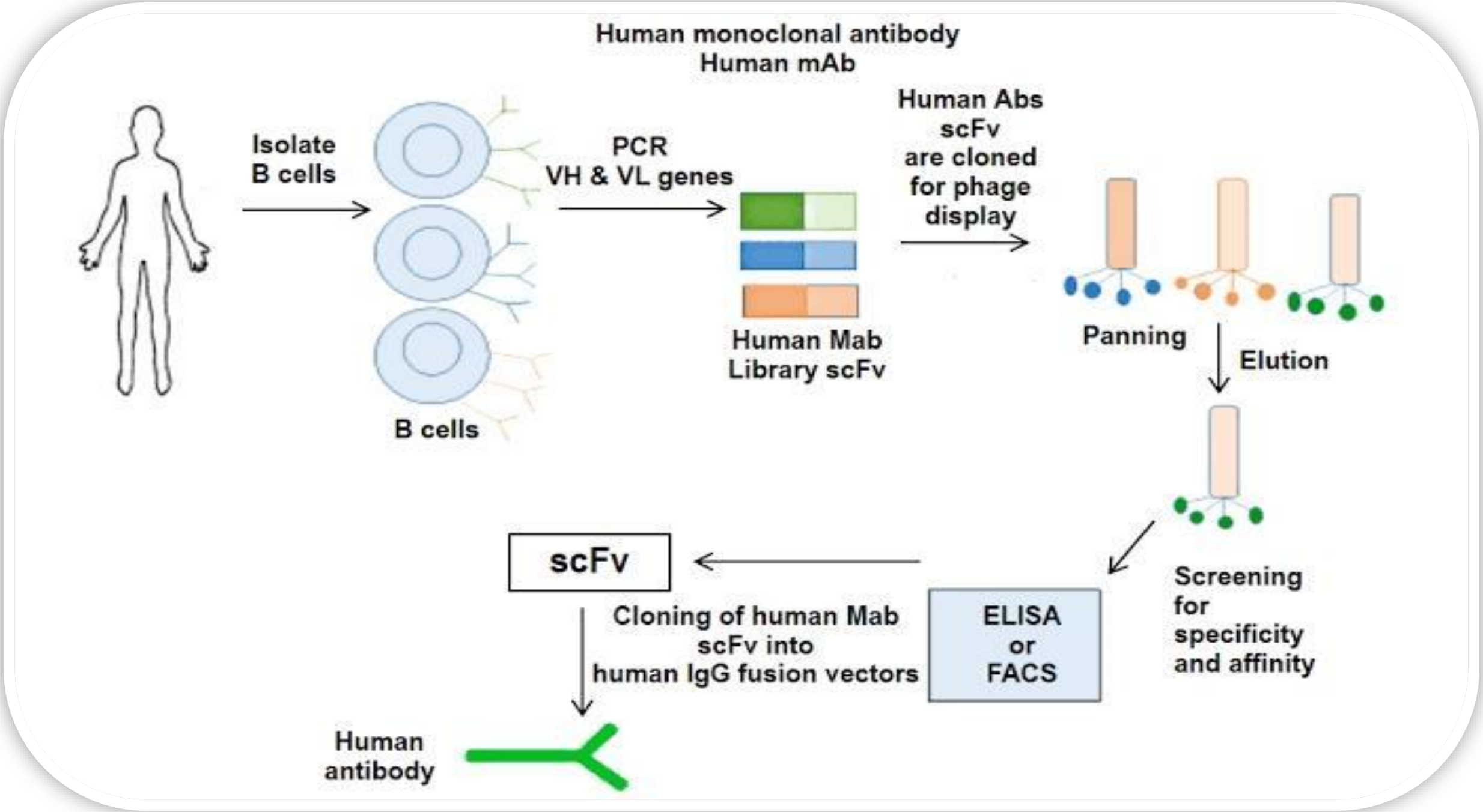


It is a combination of a human antibody with a small part of a mouse or rat monoclonal antibody. The mouse or rat part of the antibody binds to the target antigen, and the human part makes it less likely to be destroyed by the body's immune system.

Fully Human Antibodies:

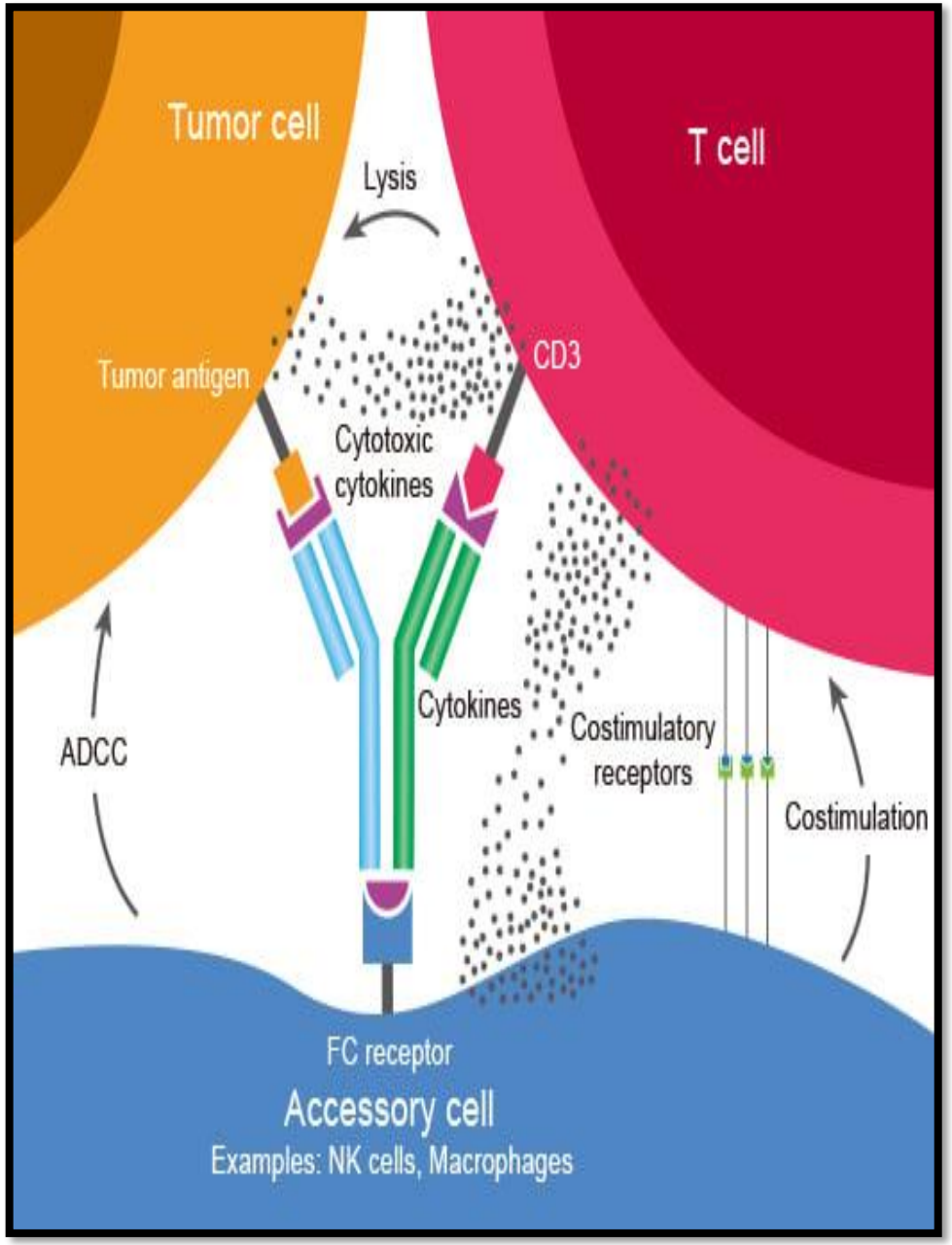
-
- **Entirely human, produced through transgenic mice or phage display libraries.**
- **Lowest immunogenicity, making them the most desirable for human therapeutic applications.**
- **Example: Adalimumab (Humira), used for autoimmune disorders**

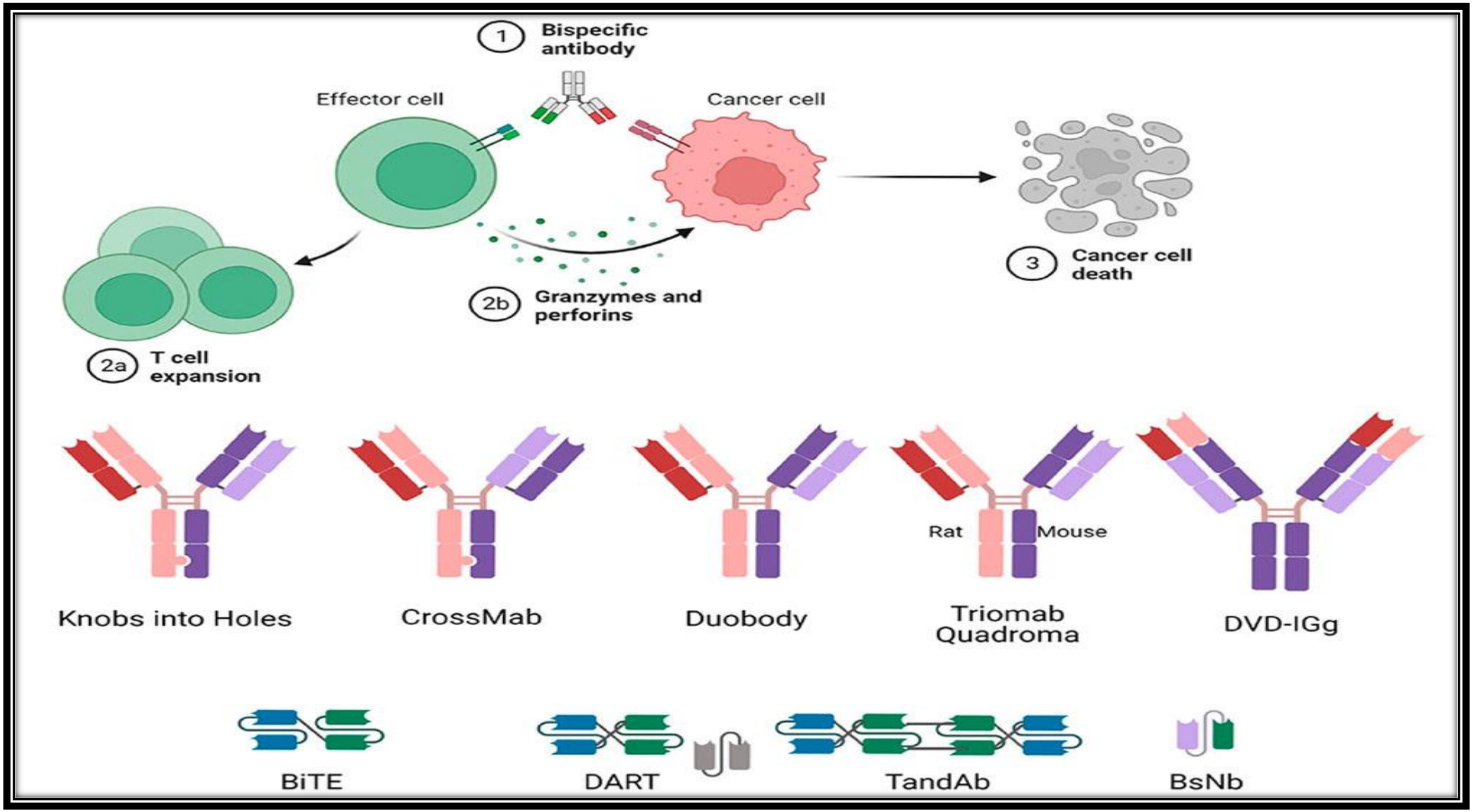



Human monoclonal antibody
Human mAb
Isolate
B cells
B cells
PCR
VH & VL genes
Human Mab
Library scFv
Human Abs
scFv
are cloned
for phage
display
Panning
Elution
Screening
for
specificity
and affinity
ELISA
or
FACS
scFv
Cloning of human Mab
scFv into
human IgG fusion vectors
Human
antibody

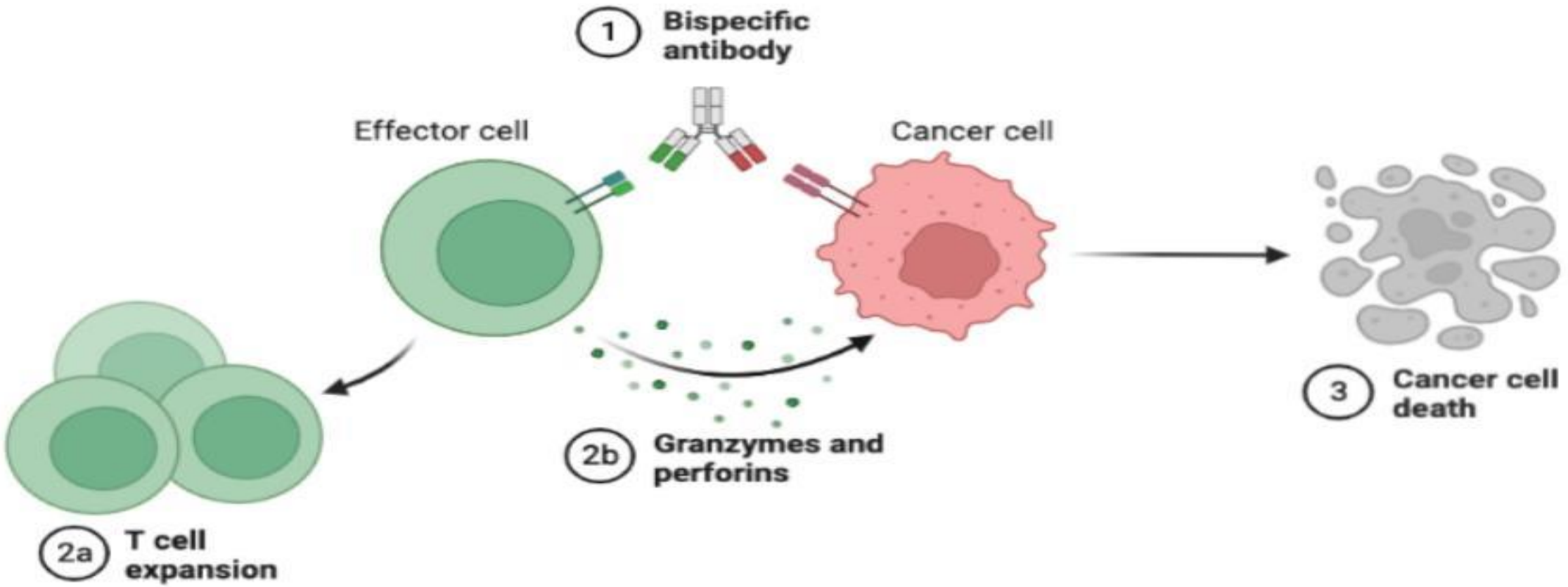
Bispecific Antibodies:

- Designed to bind two different antigens or epitopes simultaneously.
- Useful for targeted therapy (e.g., directing immune cells to cancer cells).
- Example: Blinatumomab, used in leukemia.



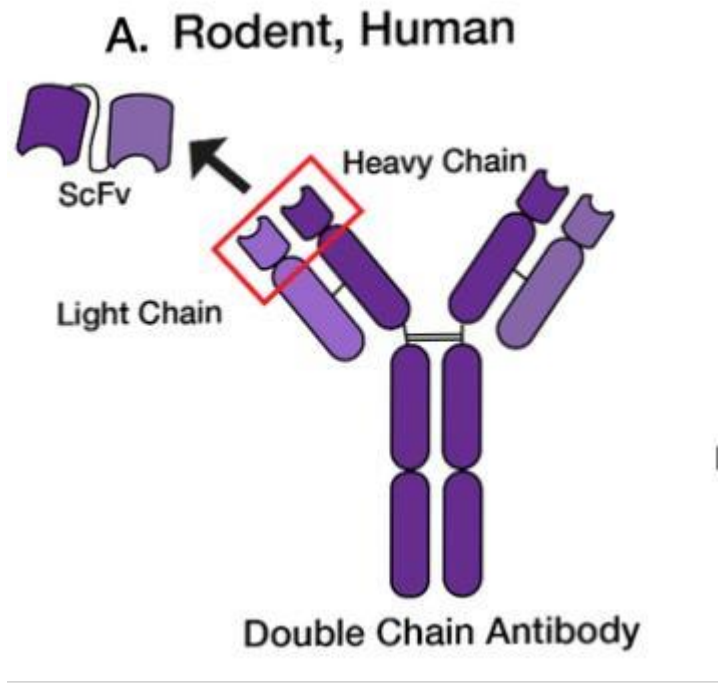

1 Bispecific antibody
Effector cell
Cancer cell
3 Cancer cell death
2b Granzymes and perforins
2a T cell expansion
Knobs into Holes
CrossMab
Duobody
Rat
Mouse
Triomab Quadroma
DVD-IGg
BiTE
DART
TandAb
BsNb

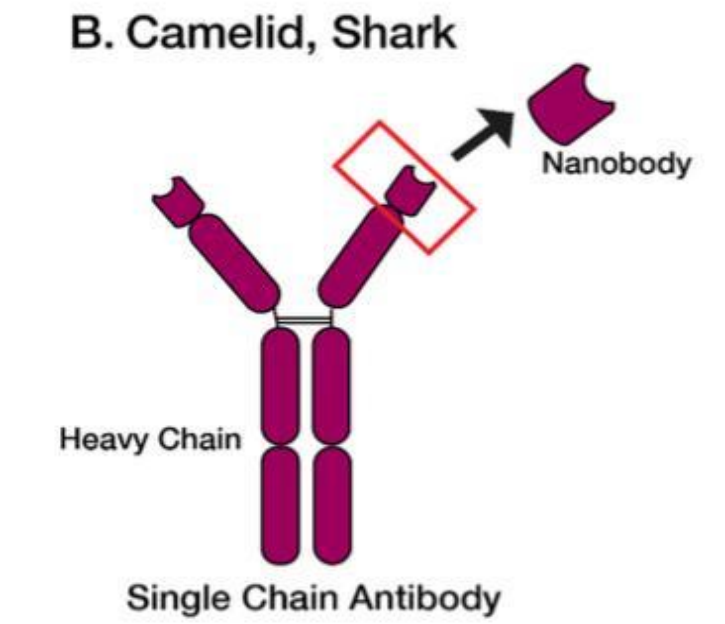
Bispecific Antibody Mechanism of Action



Antibody Fragments:

- Smaller versions of antibodies such as Fab (Fragment antigen-binding) and scFv (single-chain variable fragment).
- Retain the antigen-binding ability but are smaller and more versatile.
- Can penetrate tissues better than full-size antibodies.





PRODUCTION AND TECHNIQUES

Phage Display Technology:

- **A library of antibody fragments is expressed on the surface of bacteriophages.**
- **Phages displaying fragments that bind the target antigen are selected and amplified.**
- **Used to generate human antibodies without the need for immunizing animals.**
- **Recombinant DNA Technology:**
- **Genes encoding the desired antibody are inserted into host cells (e.g., CHO cells).**
- **The host cells produce large quantities of the antibody through cell culture.**
- **This method allows for precise control over antibody structure and modifications.**
- **Applications of Genetically Engineered Antibodies**

Transgenic Animals:

- Mice or other animals are genetically modified to produce human antibodies.
- Mice with inactivated mouse antibody genes and human immunoglobulin genes are immunized with the target antigen.
- Antibodies produced are fully human.

Hybridism Technology:

- Mice are immunized with an antigen, and their B cells are fused with myeloma cells to create hybridoma cells.
- These cells produce large quantities of monoclonal antibodies.
- Initially used to generate mouse antibodies, now adapted for humanized antibody production.

Applications of Genetically Engineered Antibodies

- **Cancer Therapy:**
- **Antibodies can target specific tumor antigens, helping to destroy cancer cells or deliver cytotoxic agents directly to the tumor.**
- **Example: Cetuximab targets the EGFR receptor in colorectal cancer.**
- **Autoimmune Diseases:**
- **Antibodies are used to neutralize overactive immune components, such as TNF-α or IL-6, which cause inflammation.**
- **Example: Infliximab and adalimumab, both targeting TNF-α in rheumatoid arthritis.**

- **Infectious Diseases:**
- **Used to neutralize pathogens like viruses and bacteria.**
- **Example: Palivizumab, used for preventing respiratory syncytial virus (RSV) infection in high-risk infants.**
- **Diagnostic Tools:**
- **Antibodies are used in ELISA, Western blotting, and immunohistochemistry to detect the presence of specific biomolecules.**
- **Fluorescent or enzyme-linked antibodies can identify and quantify target antigens**

ABZYMES

- **Abzymes (antibody enzymes) are antibodies that exhibit enzymatic activity. These antibodies are designed to bind and catalyze specific reactions like enzymes.**
- **They are artificial constructs generated by combining the specificity of antibodies with the catalytic properties of enzymes.**

MECHANISMS

- **Abzymes work by stabilizing the transition state of a chemical reaction, similar to how enzymes lower the activation energy. This allows them to catalyze the conversion of substrates into products.**

SOURCES

- **Abzymes are usually artificial constructs.**
- **They also obtained from human and animal serum.**
- **Found in normal humans and patients with autoimmune diseases**
- **These are capable of hydrolyzing proteins, DNA, RNA, polysaccharides, etc.**

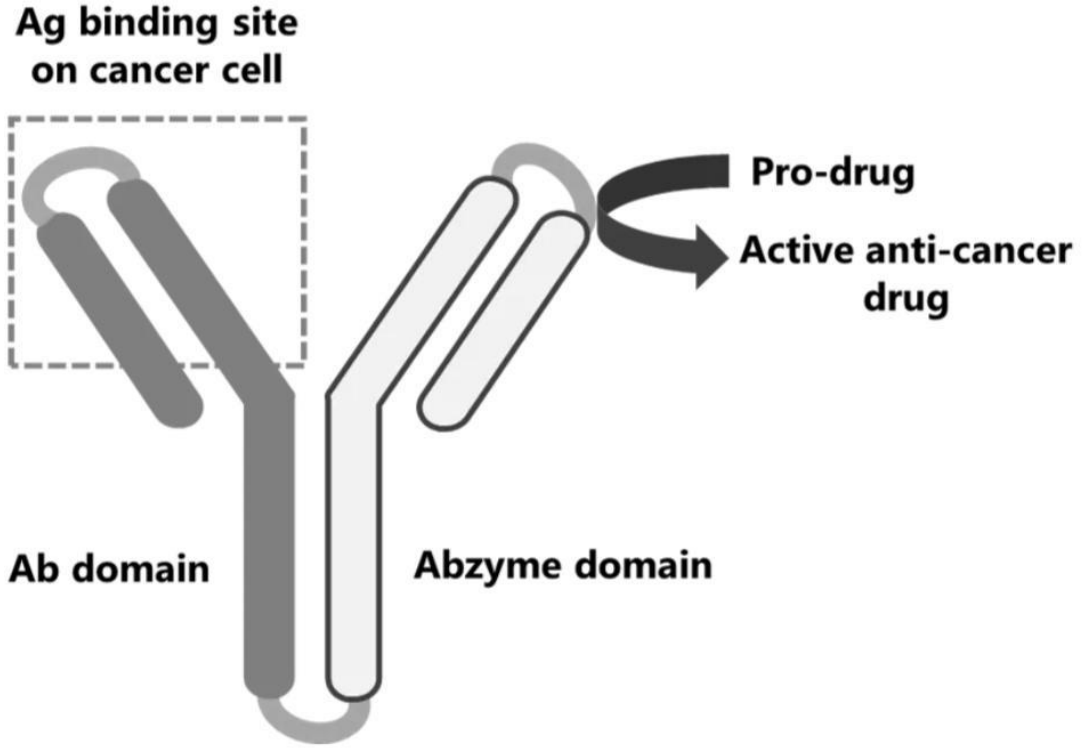


Fig 5: Abzyme in activating anti-cancer drug at the target site

APPLICATION

- **Therapeutics: They can potentially target and degrade harmful compounds or pathogens, offering a new approach in drug development.**
- **Biosensors: Abzymes can be used in biosensors for detecting specific substances due to their high specificity and ability to catalyze reactions.**
- **Prodrugs: Abzymes can be designed to activate prodrugs (inactive forms of drugs), enabling site-specific drug release.**