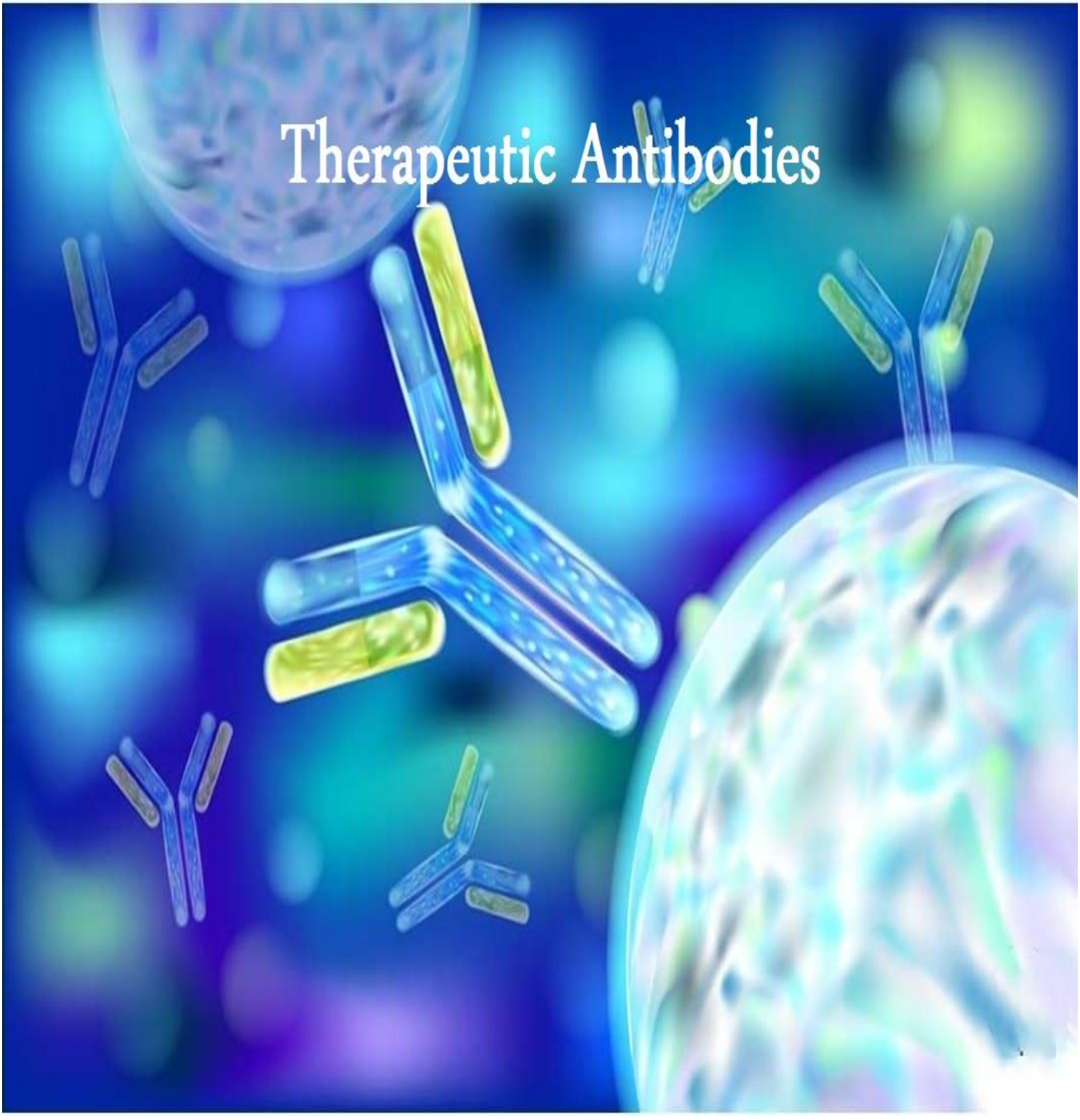
Therapeutic Antibodies

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Therapeutic proteins

* Single type of Antibody use to treat cancer can generate billion of dollars.

-Antibodies : are proteins produce by B cell cells.

-B cells they can recognize pathogens inside our bodies.

-B cells type of immune system cells.

- There job are produce anti – bodies These antibodies are bind to virus , Bacteria or toxic.

-We have antigen and anti body

-Antibody : protein have Y shape, any substance that can bind to Antibody is called antigen.

-Antigen could be protein on the surface of Bacteria or it could be protein on the surface of viruses.

-For example Corona virus vaccine after take it will stimulate B cells to produce antibodies go and bind to virus.

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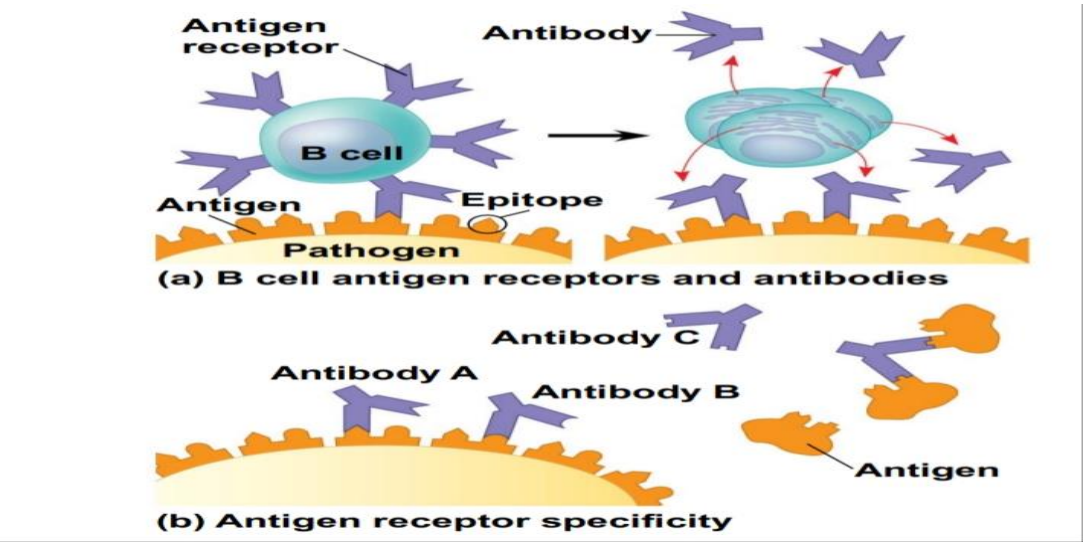
-Some antigens found in the surface of pathogens , some of antigens are soluble like Toxic substances



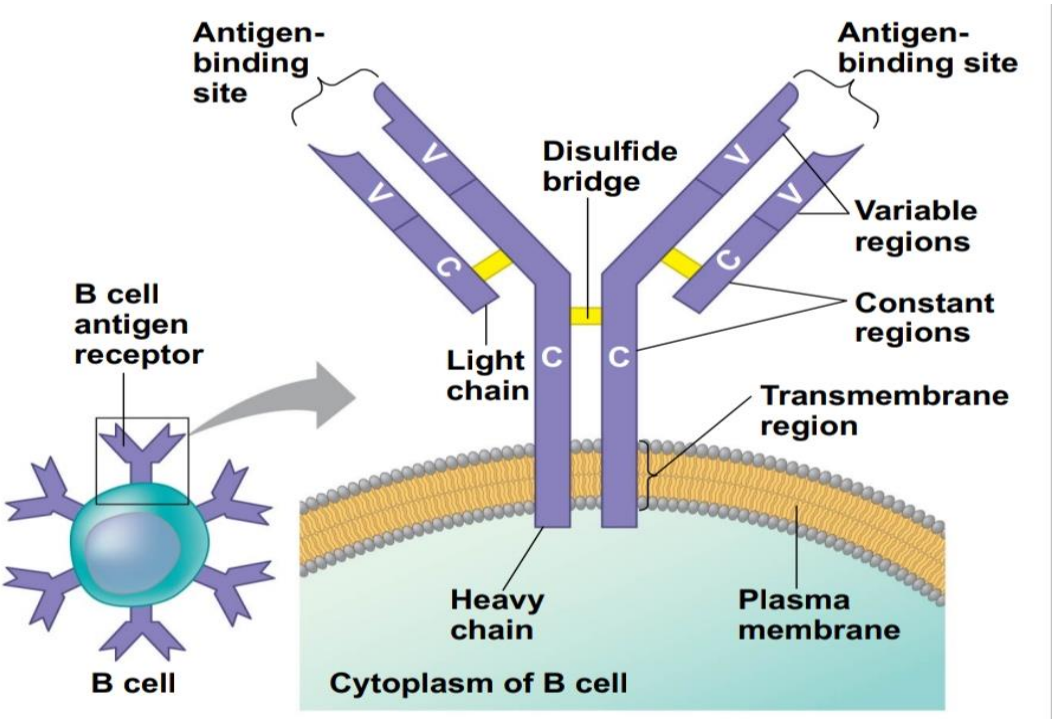
-Antibody consist of four poly peptides chains :

-Two light chains.

-Two heavy chains.

-

-The Antibody it will be first a receptor on the surface of B cells before the antigen bind to receptors on the B cells , after antigen bind to receptor bind to antigen it will stimulate B cells activated so instead B cells have antigen receptors it start secretion of soluble anti bodies.



-Each anti body has tow binding sites.

-Four chains connect to each other by disulfide bridges.

-Polypeptide consist of Amino acids, as we know there are 20 types of amino acids.

-Each amino acid consist of

- Amino group (NH2)

-

•Carboxyl group (COOH)

•Side chain (R group)

-All amino acids have the same Carboxyl Group and Amino Group but different in R Group.

-One type of amino acids called Cysteine has SH on the side Chain.

-TOW Cysteine amino acids from different polypeptides can oxidize to form disulfide bridge and join These tow polypeptides to gather

-Each chain consist of TOW region : constant region, and variable region.

-Every B cell have the same amino acids in constant region

-Every B cell has unique amino acids in variable region, which mean it form antigens binding site.

-So every B cell binding different antigen, because there different amino acids in variable region from B cells to other B cells.

-Antigen binding site consist form amino acid from variable region light chain and from amino acids from variable region heavy chain.

-With in each variable region have three important loops.

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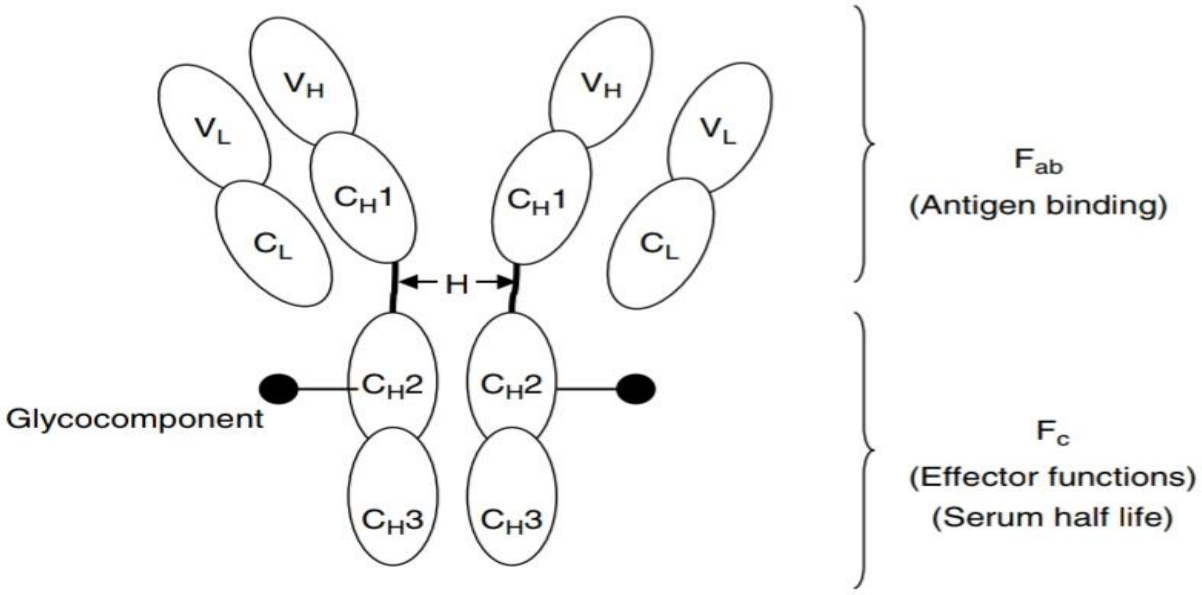
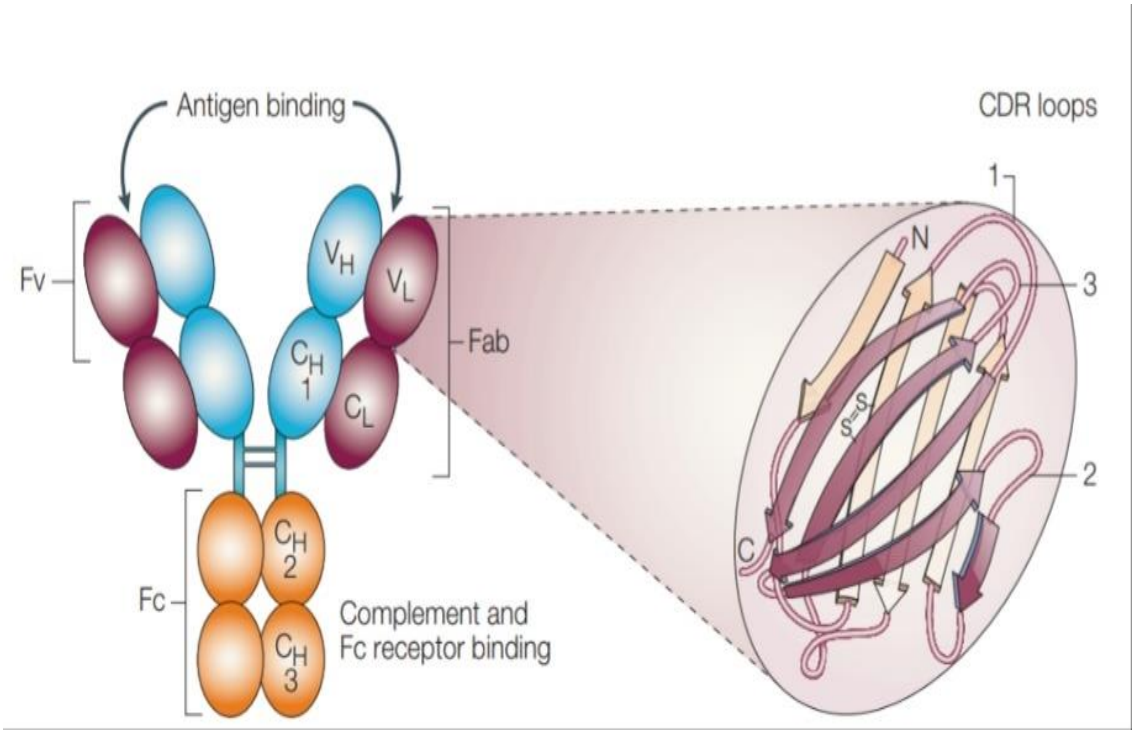
-Most the secondary structure in variable region are made from. Beta sheets.



-These B sheets connected by loops These loops called CDR loops.

CDR : complementary determining region.



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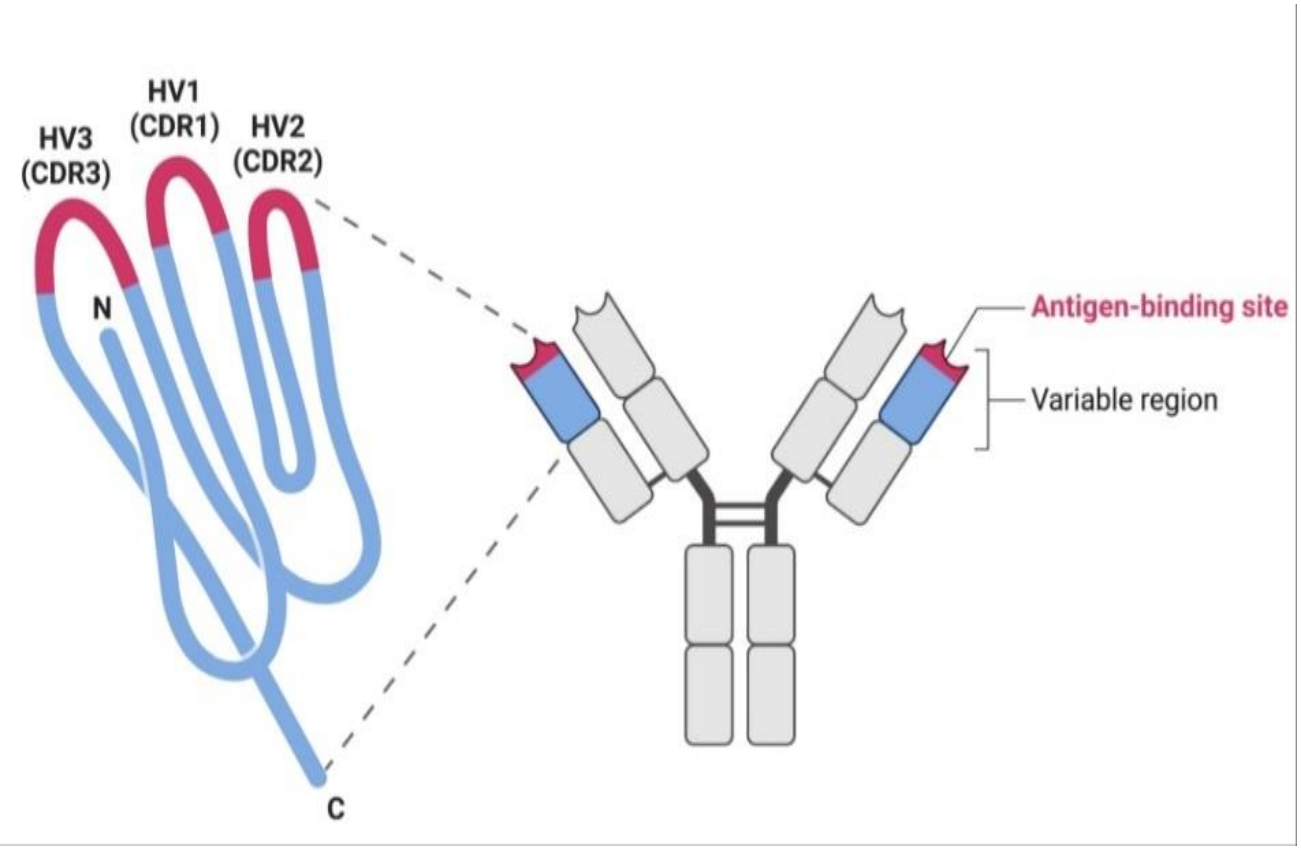
Antigen bind exactly in CDR

With in the antigen binding site there are three Hyper variable, amino acids in These loops are different in Antigen receptor in different B cells this why every antibody can bind in one antigen.

Five types of antibodies, Ig stand for immunoglobulin Another Name of Antibody IgA, IG, Ig D, Ig M, Ig E

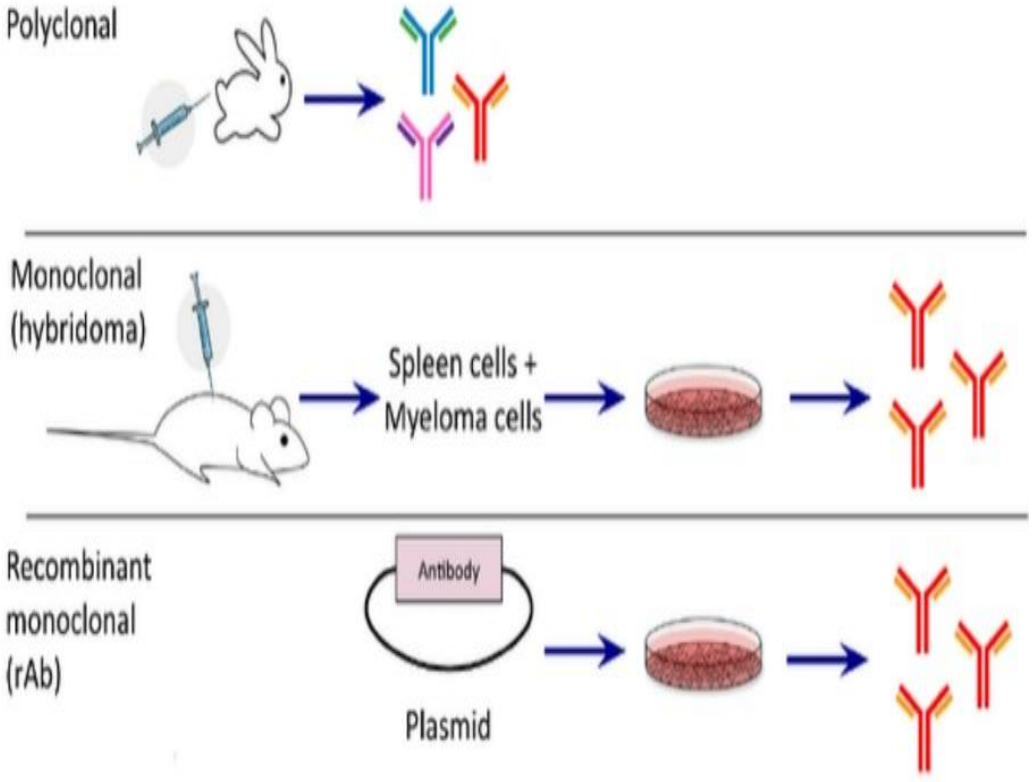


Polyclonal Antibody

When B cell activated they start dividing by mitosis to produce clone
(Cell cloning).

-Also we have monoclonal antibody what the differences between them?

-



-Monoclonal means we have solution of anti bodies all of These anti bodies bind to the same antigen.

-Polyclonal anti bodies we have solution of antibodies bind to different antigens, each anti body come from different cell.

-We get antibodies from our blood and lymphatic system.

-If we get anti bodies from animal it called anti serum.

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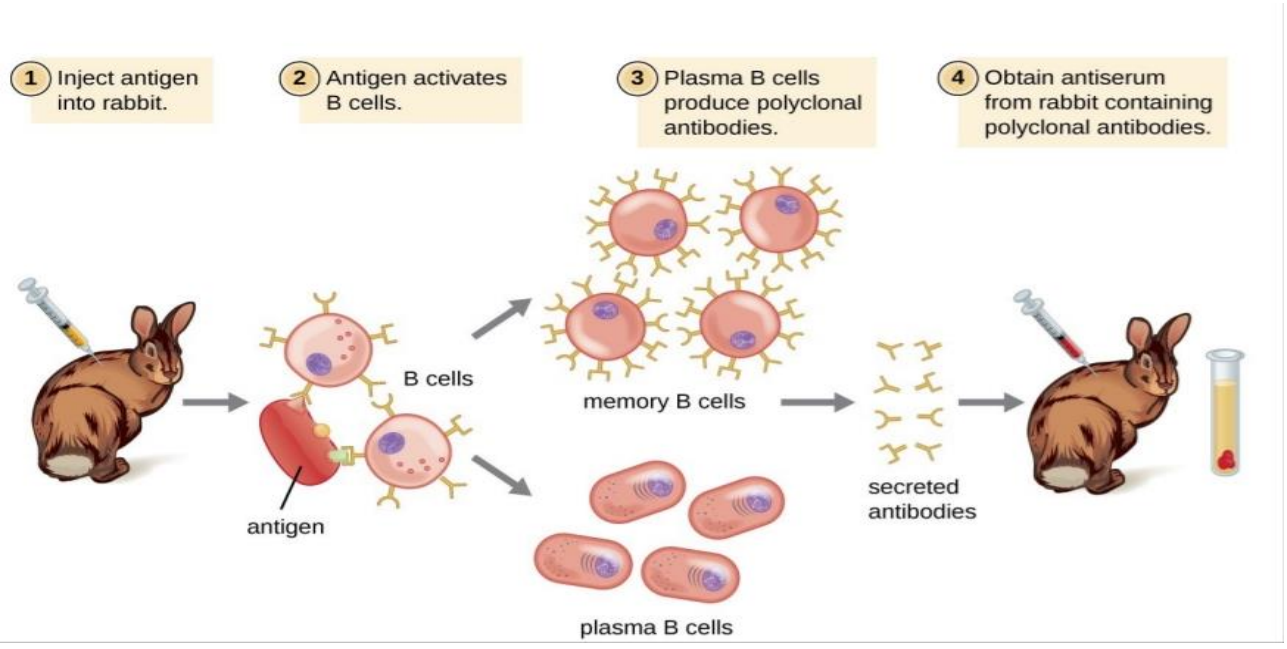
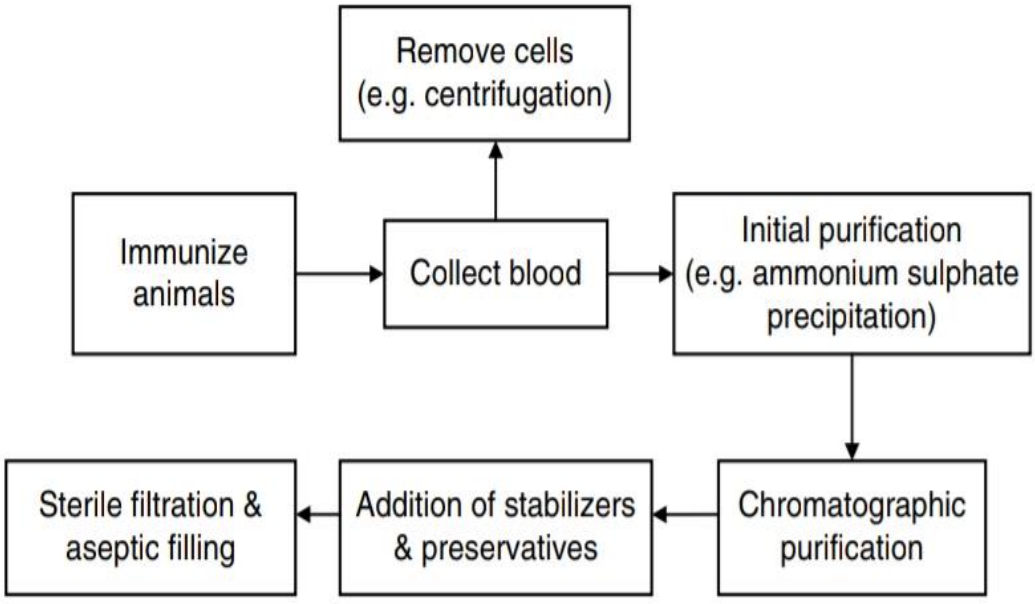
-Blood consist of cells and other Component if we take the sample of blood and do centrifugation we got two Layers.

- Cell
- Plasma





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-Inside the plasma we found serum, the serum is the plasma without clotting factors.

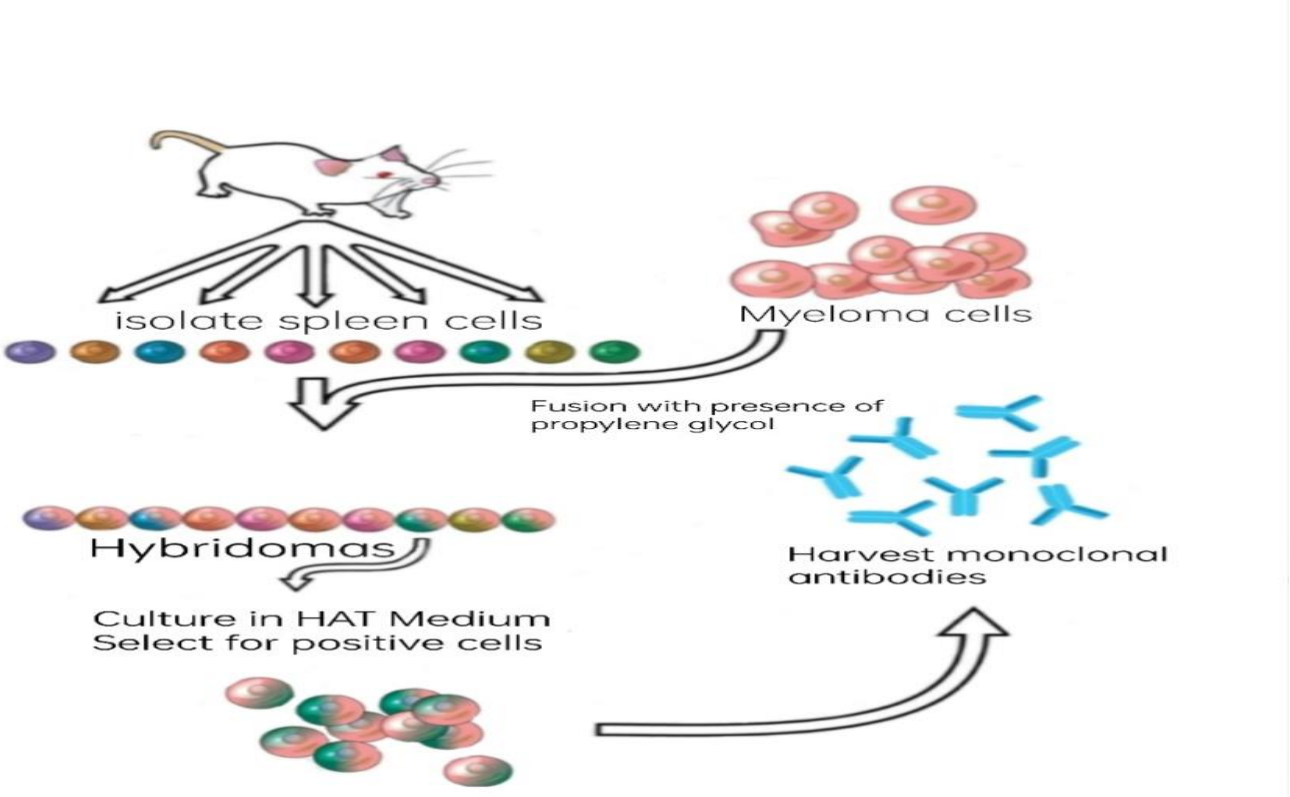
-Anti serum : Anti bodies in serum.

-Anti bodies we take from blood of animal are called anti serum.

-Antibodies take from blood of human called immunoglobulin preparations.

-We can produce Polyclonal antibodies By inject antigen to animal.

-We can produce monoclonal antibodies By Hybridoma technology.



-We can produce monoclonal antibodies By Hybridoma technology.

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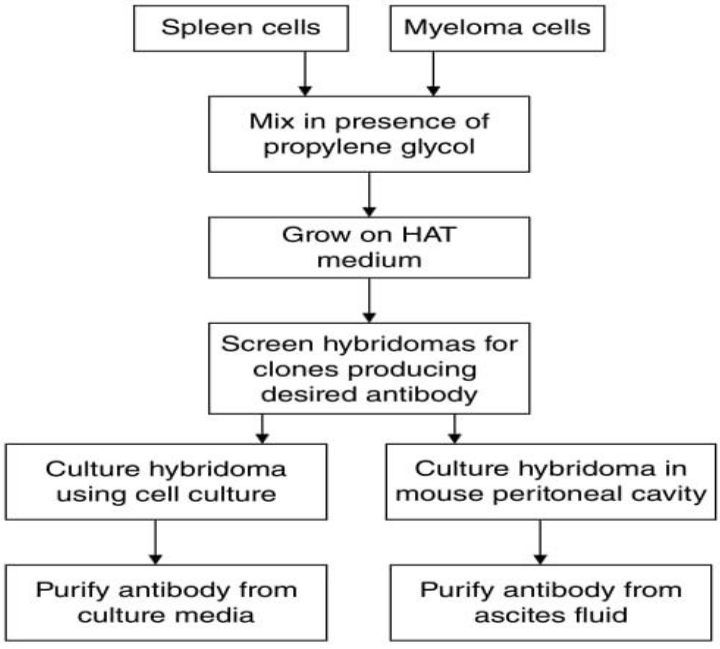


-We can use monoclonal and polyclonal antibodies In treatment and in other applications.

-When we inject antigen into animal body, the antigen will stimulating Antibodies.

-

Table 7.1 Polyclonal antibody preparations most commonly used to induce passive immunity.

Antibody preparation	Source	Antibody specificity
Normal immunoglobulin	Human	Exhibits a wide range of specificities against pathogens which are prevalent in the general population
Hepatitis B immunoglobulin	Human	Antibodies exhibiting a specificity for hepatitis B surface antigen
Measles immunoglobulin	Human	Antibodies exhibiting a specificity for measles virus
Rabies immunoglobulin	Human	Antibodies exhibiting a specificity for rabies virus
Cytomegalovirus immunoglobulin	Human	Antibodies exhibiting a specificity for cytomegalovirus
Varicella zoster immunoglobulin	Human	Antibodies exhibiting a specificity for the causative agent of chickenpox
Tetanus immunoglobulin	Human	Antibodies exhibiting a specificity for the toxin of *Clostridium tetani*
Tetanus antitoxin	Horse	Antibodies raised against the toxin of *Clostridium tetani*
Botulism antitoxin	Horse	Antibodies raised against toxins formed by type A, B or E *Clostridium botulinum*
Diphtheria antitoxin	Horse	Antibodies raised against diphtheria toxin or toxoid
Gas gangarene antitoxins	Horse	Antibodies raised against the α-toxin of *Clostridium novyi*, *C. perfringens* or *C. septicum*
Scorpion venom antisera	Horse	Antibodies raised against venom of one or more species of scorpion
Snake venom antisera	Horse	Antibodies raised against venom of various poisonous snakes
Spider antivenins	Horse	Antibodies raised against venom of various spiders, in particular the black widow spider

-***This is called Active immunization***

-Passive Immunization : we inject antibodies directly not antigen to give a animal immunity, we not stimulate B cell.

-For examples : During pregnancy the embryo dose not has immune system so he take anti bodies from mother.

Also after he born he take anti bodies from mother by her milk.

-

-*This called Passive immunity*

-We can produce antibodies By use animal and we can use in Passive immunity.

-Most Polyclonal antibodies use in Passive Immunization, the most monoclonal antibodies.

-Using Hybridoma require use Cancer cells called myloma cells.

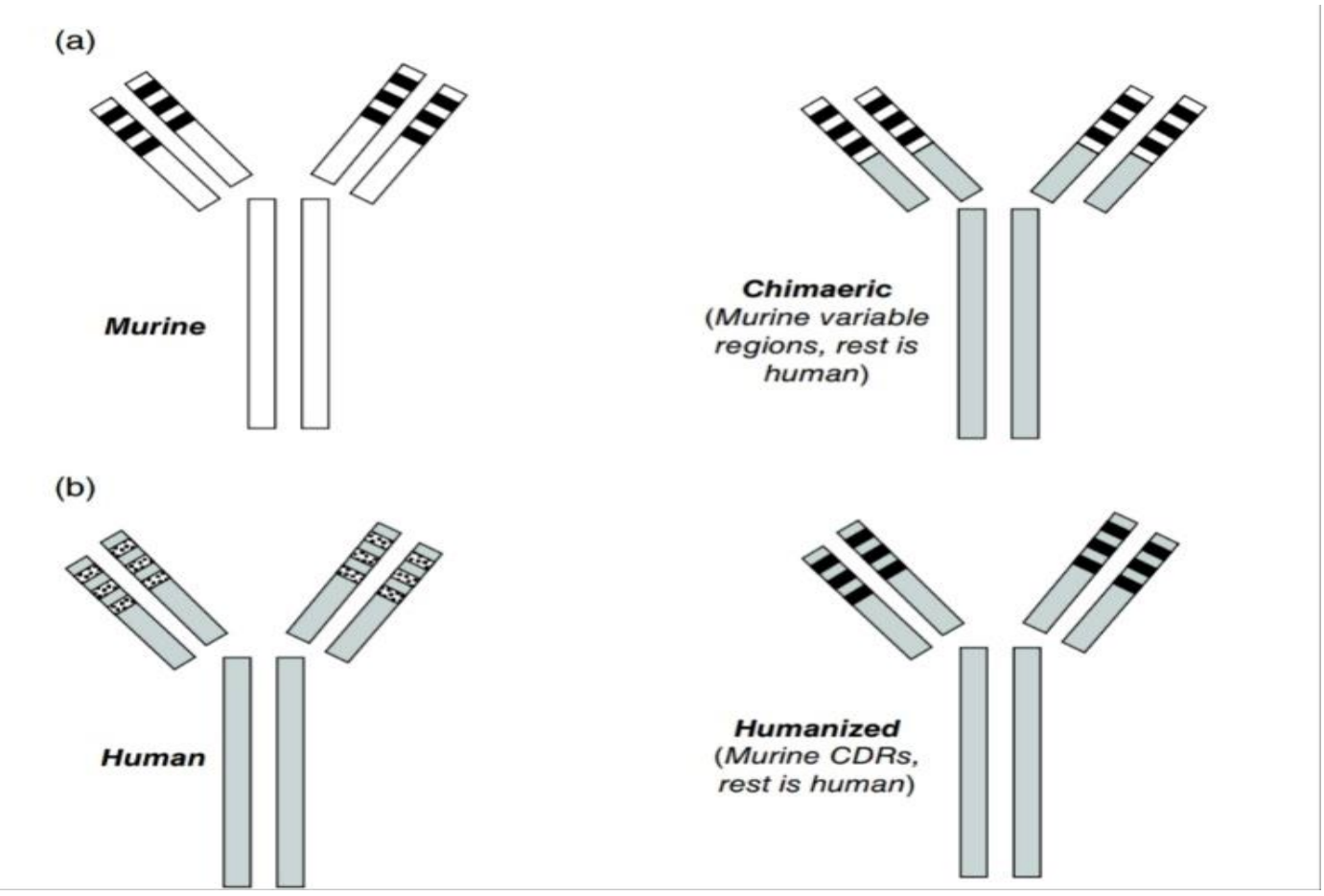
-Monoclonal antibodies mainly produce in mice

-We have Chimeric and humanized antibodies

-First generation of antibodies got from mouse so we take about mouse proteins, if we inject this protein will lead to problem because the body this protein will Consider as foreign molecules.

-This why first generation of anti body not use in treatment, if use to Research.

-



*Murine : MOUSE

-In Chimeric antibodies the V region from the mouse Another parts from Human proteins

-If we inject Chimeric antibody to human it can cause small immunological reactions.

-

-Also we have humanized anti bodies the variable region from Mouse and human and other parts from Human.

-Chimeric and humanized are types of Monoclonal antibodies and

Product	Company	Indication	Approved
Actemra/RoActemra (tocilizumab). Humanized Mab specific for IL-6. Produced in a CHO cell line	Genentech/Roche	Rheumatoid arthritis	2010 (USA) 2009 (EU)
Adcetris (brentuximab vedotin). Chimeric Mab conjugate specific for human CD30 (expressed on the surface of lymphoma cells). Produced in a CHO cell line	Seattle Genetics Inc.	Lymphoma	2011 (USA)
Avastin (bevacizumab). Humanized Mab raised against vascular endothelial growth factor. Produced in a CHO cell line	Genentech/Roche	Carcinoma of the colon or rectum, metastatic breast cancer	2004 (USA) 2005 (EU)
Erbitux (cetuximab). Chimeric Mab raised against human EGF receptor. Produced in a Sp2/0 cell line	ImClone Systems/ Bristol-Myers Squibb	Treatment of EGF receptor-expressing metastatic colorectal cancer	2004 (USA, EU)
Herceptin (trastuzumab). Humanized Mab directed against human epidermal growth factor receptor (HER)2. Produced in a murine hybridoma cell line	Genentech/Roche	Treatment of metastatic breast cancer if tumour overexpresses HER2 protein	1998 (USA) 2000 (EU)
MabCampath (EU) or Campath (USA) (alemtuzumab). Humanized Mab directed against CD52 surface antigen of B lymphocytes. Produced in a CHO cell line	Genzyme	Chronic lymphocytic leukaemia	2001 (EU, USA)
Mabthera (EU) or Rituxan (USA) (rituximab). Chimeric Mab directed against CD20 surface antigen of B lymphocytes. Produced in a CHO cell line	Roche/Genentech	Non-Hodgkin lymphoma	1997 (USA) 1998 (EU)
Mylotarg (gemtuzumab zogamicin). Humanized Mab–toxic antibiotic conjugate targeted against CD33 antigen found on leukaemic blast cells. Produced in an NS0 cell line	Pfizer/Wyeth	Acute myeloid leukaemia	2000 (USA) Withdrawn 2010

TNF, tumour necrosis factor.

-

produce by Recombinant DNA technology

-Antibodies use to treat certain Diseases some of These Diseases Cancer Disease, and Chronic Disease.

Table 7.3 Chimeric and humanized monoclonal antibodies (Mabs) that have gained approval for general medical use in the EU and/or the USA. Many are also marketed in other world regions.

Product	Company	Indication	Approved
Perjeta (pertuzumab). Recombinant humanized Mab specific for HER2. Produced in a CHO cell line	Genentech	Breast cancer	2012 (USA)
Raptiva (efalizumab). Humanized Mab that binds to the LFA-1 antigen, which is expressed on all leukocytes. Produced in a CHO cell line	Genentech, Serono	Treatment of adult patients with chronic moderate to severe plaque psoriasis	2003 (USA) 2004 (EU) Withdrawn 2009
Remicade (infliximab). Chimeric Mab directed against TNF-α. Produced in a Sp2/0 cell line	Janssen	Treatment of Crohn's disease	1998 (USA) 1999 (EU)
Simulect (basiliximab). Chimeric Mab directed against the α-chain of the IL-2 receptor. Produced in a murine myeloma cell line	Novartis	Prophylaxis of acute organ rejection in allogeneic renal transplantation	1998 (EU, USA)
Soliris (eculizumab). Humanized IgG that binds human C5 complement protein. Produced in a murine myeloma cell line	Alexion	Paroxysmal nocturnal haemoglobinuria	2007 (EU, USA)
Synagis (palivizumab). Humanized Mab directed against an epitope on the surface of respiratory syncytial virus. Produced in a murine myeloma cell line	MedImmune, Abbott	Prophylaxis of lower respiratory tract disease caused by respiratory syncytial virus in paediatric patients	1998 (USA) 1999 (EU)
Tysabri (natalizumab). Humanized Mab raised against selected leukocyte integrins. Produced in a murine myeloma cell line	Biogen Idec Inc. Elan	Treatment of patients with relapsing forms of multiple sclerosis	2006 (EU) (USA: approved 2004, suspended 2005, resumed 2006)
Xolair (omalizumab). Humanized Mab that binds IgE at the site of high-affinity IgE receptor binding. Produced in a CHO cell line	Genentech/Roche Novartis	Treatment of adults/adolescents with moderate to severe persistent asthma	2003 (USA)
Zenapax (daclizumab). Humanized Mab directed against the α-chain of the IL-2 receptor. Produced in a NS0 murine cell line	Roche	Prevention of acute kidney transplant rejection	1997 (USA) 1999 (EU) Withdrawn 2009

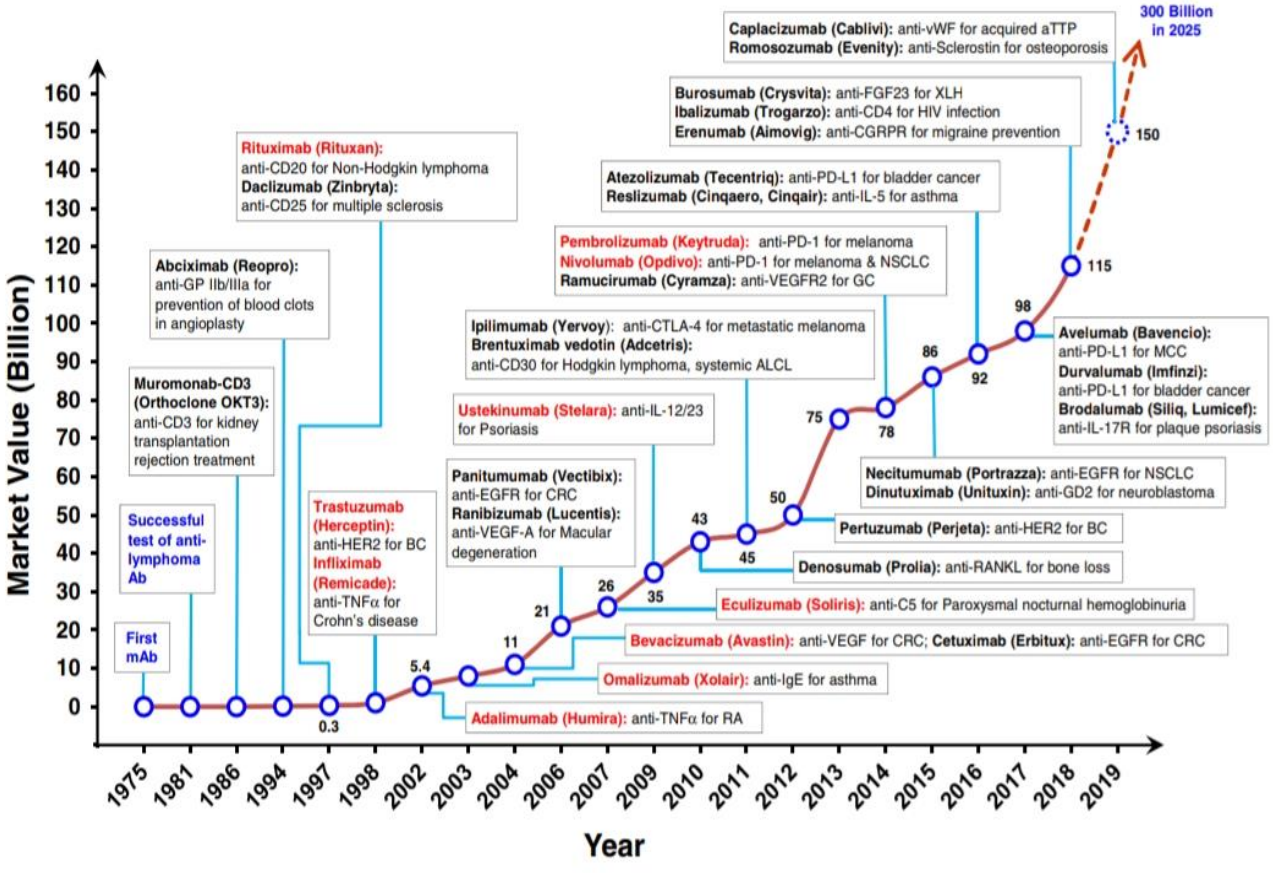
TNF, tumour necrosis factor.

-Antibodies is very cost (expensive), Companies sell antibodies By billion of Dollars.

-Many of These Drugs (Anti-bodies) are not Available to all Cancer patients.

-

-Pharmaceuticals Companies earn around 150 billion Dollars per year from sell These antibodies.



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-Fully Human Monoclonal antibodies

-Mean we need produce a Human Antibody that contain only Human Amino acids.

-Antibody is a protein encode by genes.

-We have work with Human immune globulin genes, the problem with been to find an Antibody that can bind specifically to particular antigen.

-Inside our bodies we have Millions of B cells These cells are responsible to produce Antibodies, each B cell produce specific Antibody that Can bind to one antigen.

-Hardest part to choose the B Cell produce Antibody That will bind to the specific antigen that I want.

-Most our cells contain the same genes, but These Millions B cells they have different genes to make Different antibodies, this why we Prepare immune globulin genes library.

- Immunoglobulin genes library : try to get all the genes encoding all Different Antibodies.

-Then we will do library expression : genes express to produce Antibodies then we choose the Antibody bind to specific antigen.

-

-Isolate all genes from B cells - - - -> do expression to all HIGg - - - - do selection to antibodies.

-This process could be done by tow techniques.

- Phage display
- Transgenic Mice

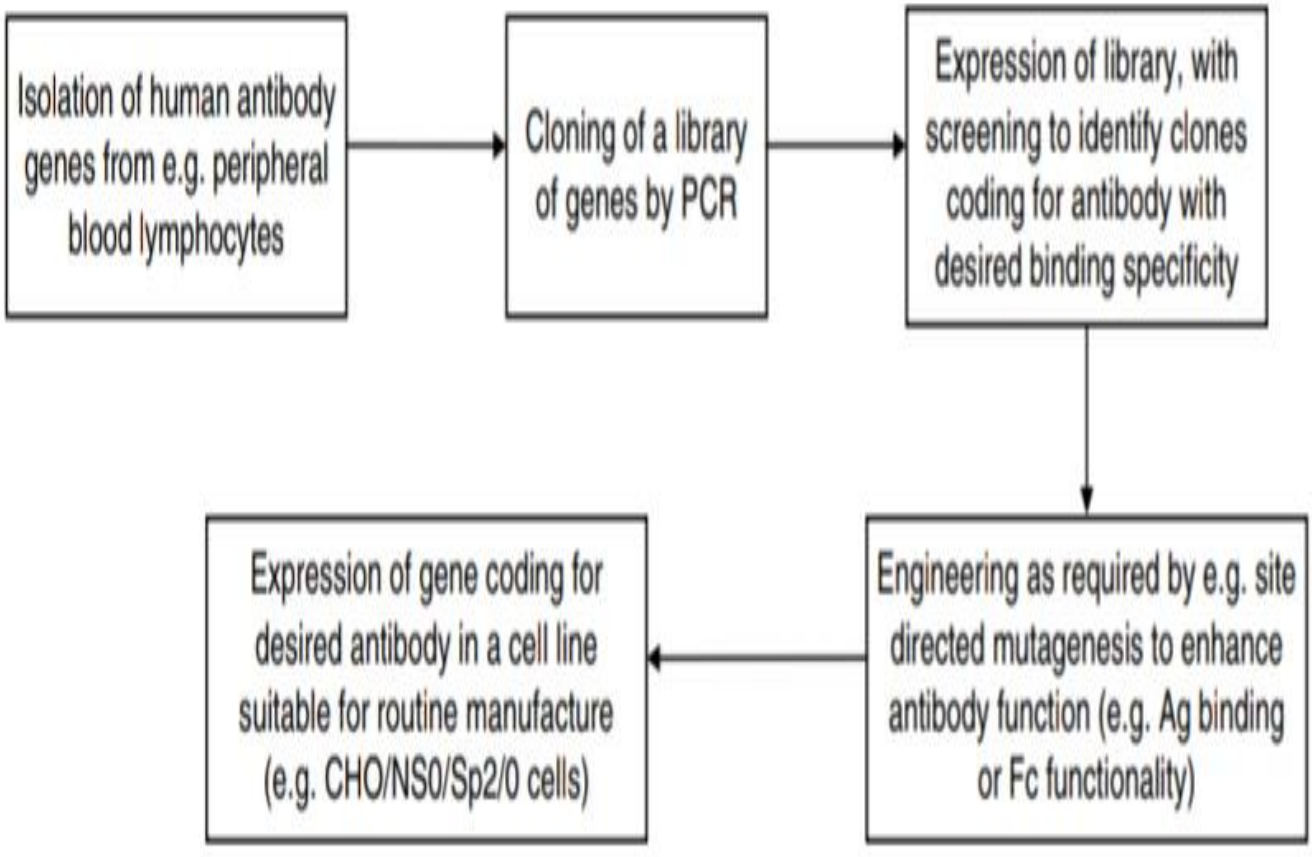

Figure 7.7 Overview of the general approach undertaken to produce fully human monoclonal antibodies. Refer to text for further details.

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-Phage display

-First isolate antibodies genes from the blood isolated B cells then clone all immunoglobulin genes by PCR.

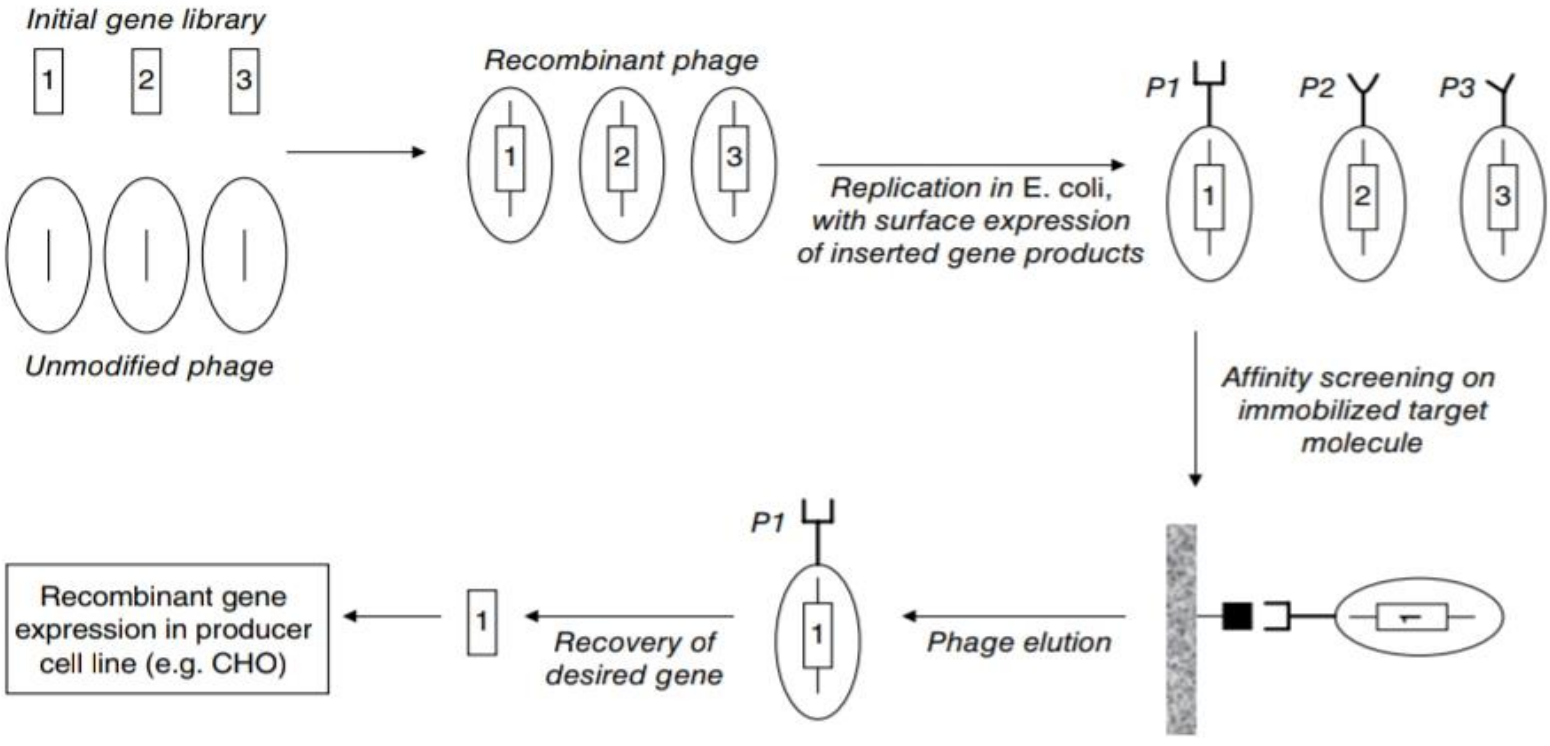
-Then we do screening to identified the antibodies.

-In phage display we use the phage to display antibodies but how?

-We have attach Antibody to antigen do now the antigen is immobilize.

-We put inside the phage immunoglobulin gene each phage get on gene now we have Recombinant phages.

-



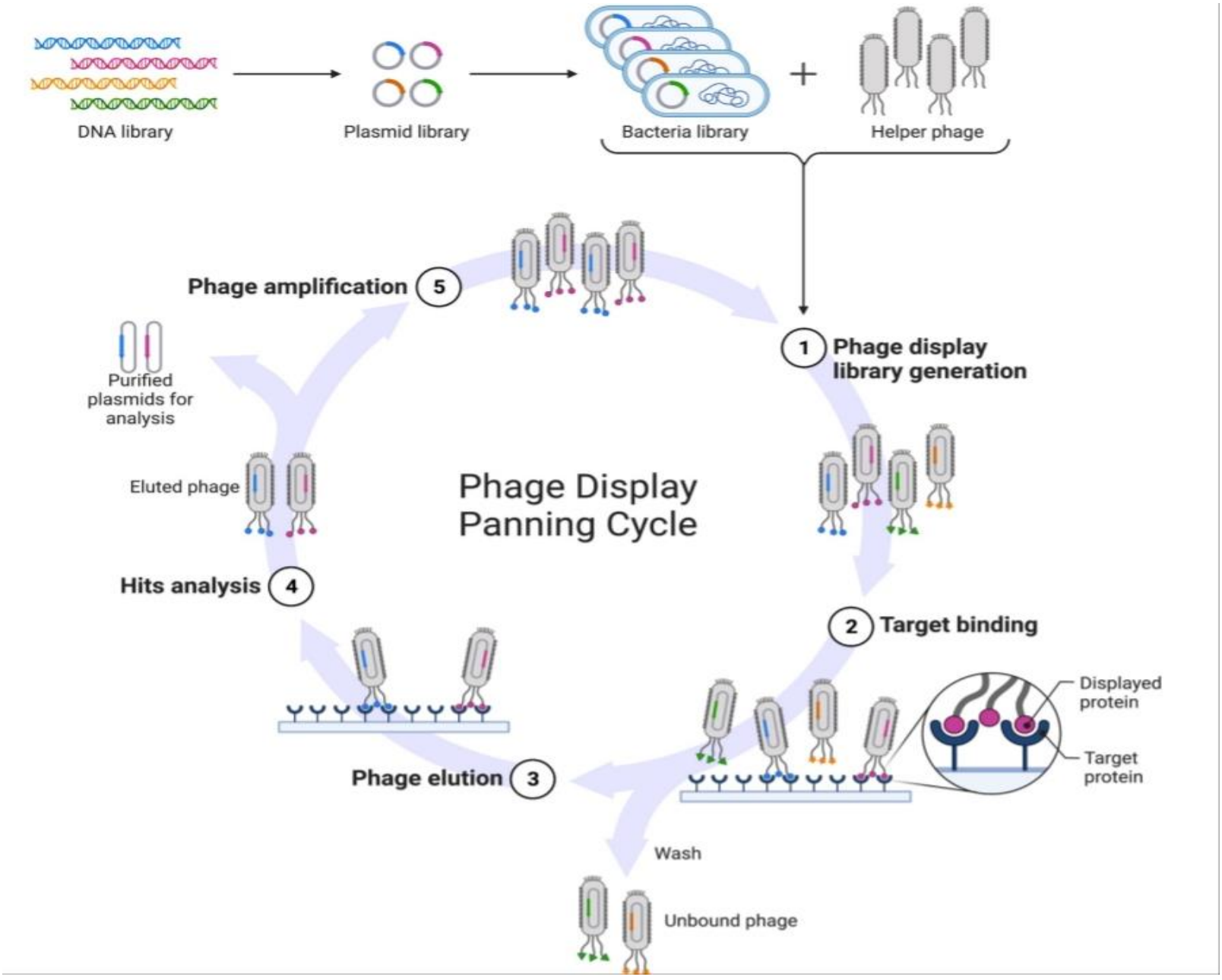
Modified from Walsh, G. (2007) *Pharmaceutical Biotechnology: Concepts and Applications*. John Wiley & Sons Ltd, Chichester.

-Then we take These phages and allow them to replicated inside E. Coli and express the gene so each phage will express Antibody.

-Then take These phages and put the on immobilized antigen..

-

-Next step is wash away all antibodies that are no bind to an antigen, and keep Antibody that bind to an antigen.



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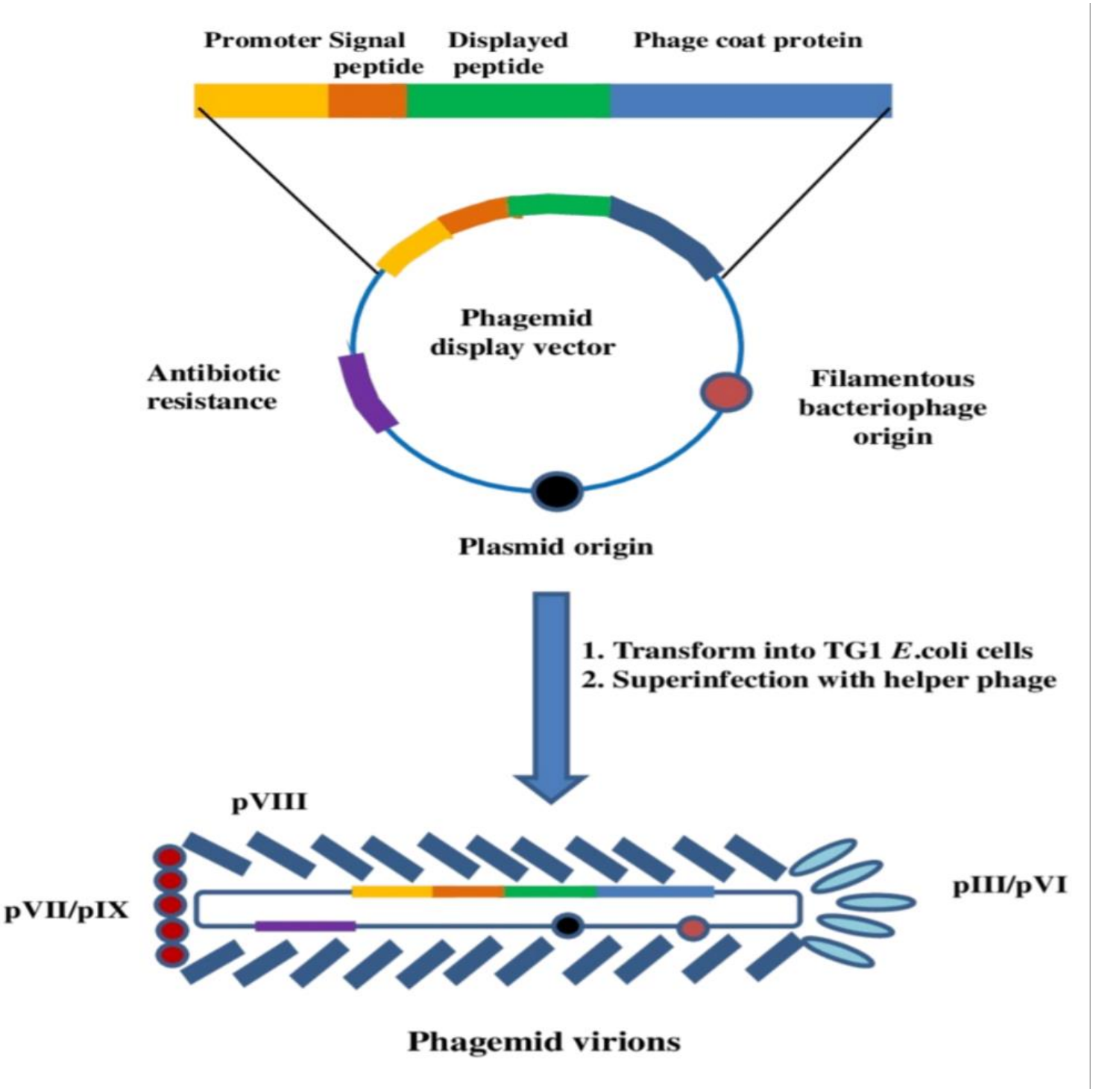
-We can use this technique of I have protein, and we want to know what is the molecules that interact with this protein.



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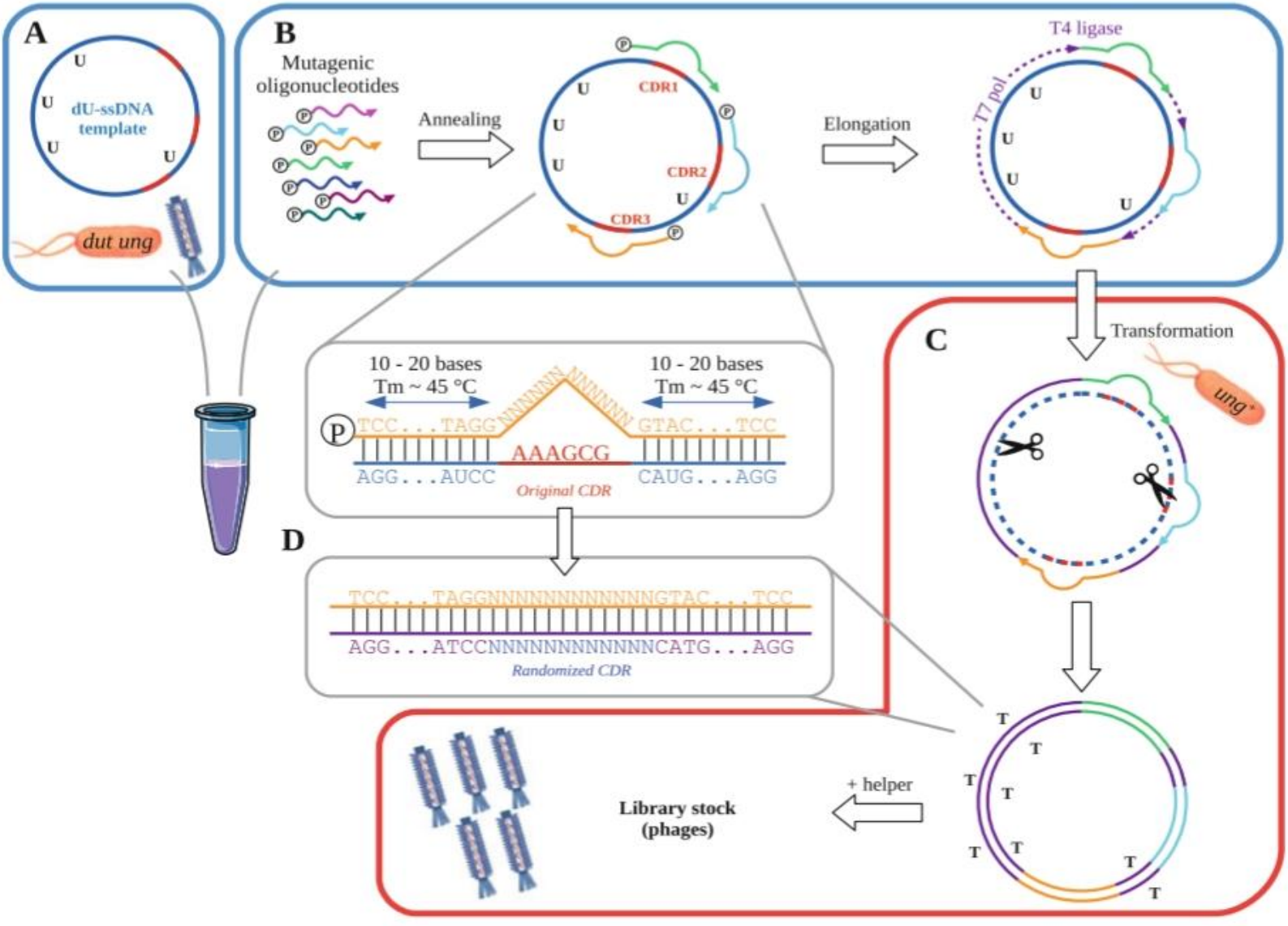
Synthetic Antibody library:

-Allow the phage display the proteins to know which protein bind in my protein.

- Display panning cycle

-Synthetic anti body library : Generate Millions of Antibodies gene without stimulate B Cells.



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-CDR : Part of Antibody very important to bind Antibody to the antigen

-CDR very small part of Antibody.

-CDR encoding by codons.

-If we are Randomly introduce mutation in the CDR we Can generate different antigen binding sites.

-Apply Random Mutagens to generate Millions different CDR To generate different Millions antibodies.
-In all antibodies they have the same Constant regions but different Variable regions.

-We can clone constant region on plasmid and introduce Random

Mutagens in CDR region.

-We use mutagen primer to bind in CDR to start introduce Random Mutagen.

-We have design the primer to bind after and before the CDR but in the middle we add degenerate primers it done by PCR.

-We have Hundred of primer that have the same sequence after and before CDR but different sequences in the middle.

-Then we take this plasmid and put them inside phages and do phage display.

-

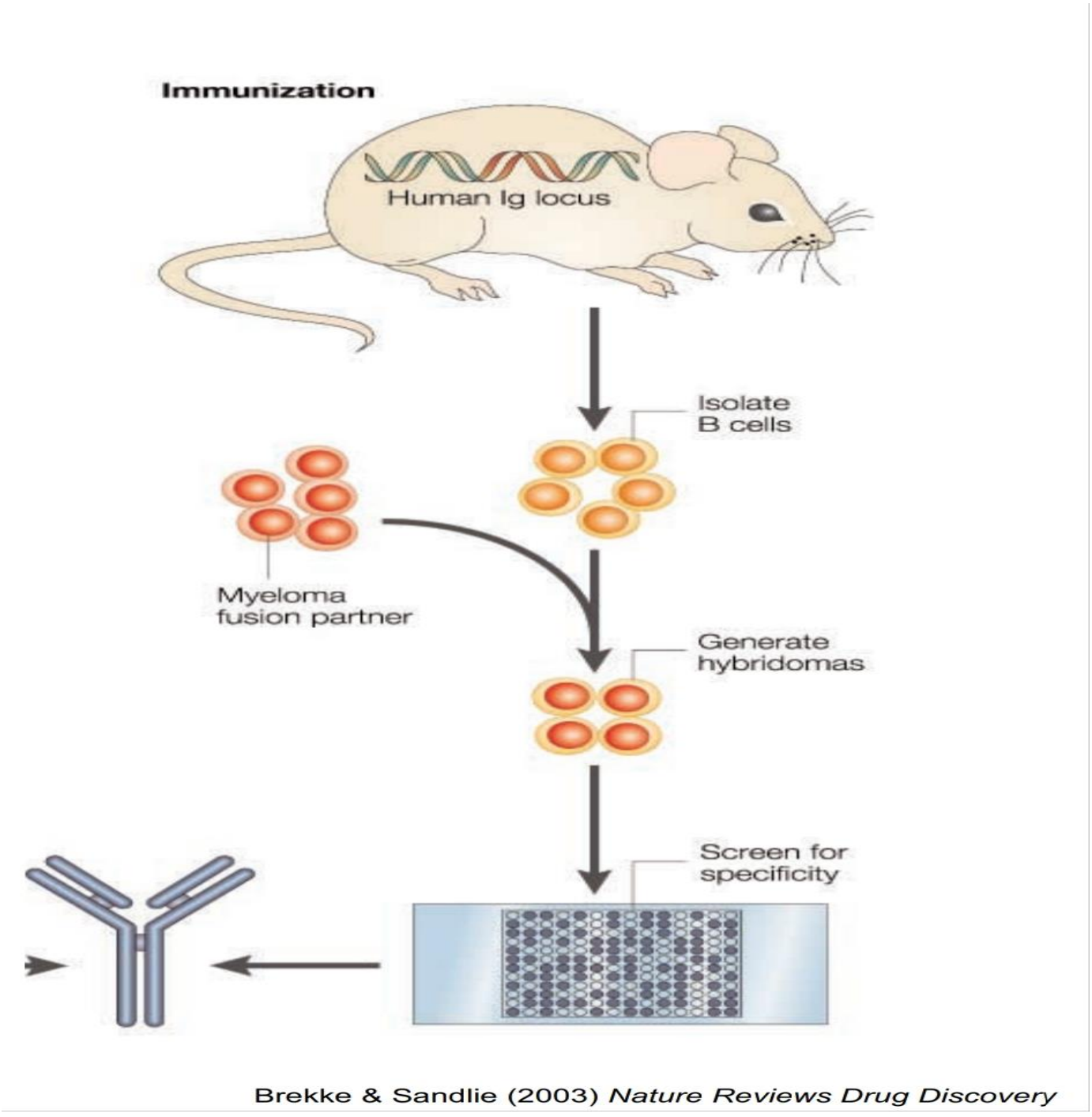
-Transgenic Mouse

-We take immunoglobulin genes from mouse and replace it by Human immunoglobulin Gene.

-After it done now the mouse will produce Human Antibodies NOT mice antibodies.

-Immunization : inject mouse by our Antigen, the mouse must be transgenic Mouse.

-



-Xenomouse : produce by removing or inactivate immunoglobulin genes from mouse.

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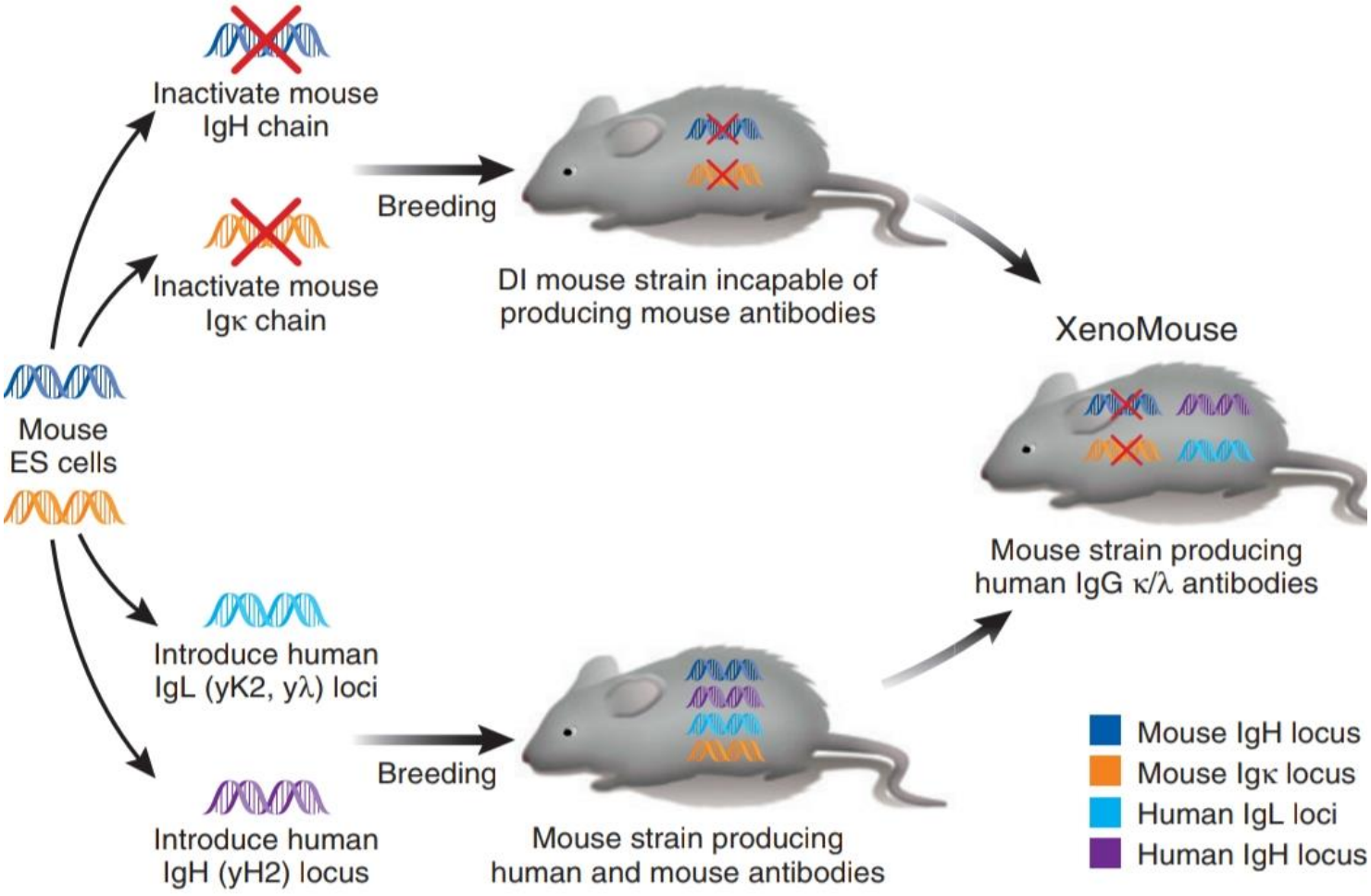
-We can inactivate mouse immunoglobulin genes by use embryonic stem cells.



-Then we introduce Human immunoglobulin genes

-Now we have two genes for mouse and two

human genes.

-Then we breed two mouse to generate transgenic Mouse.

- Therapeutic Applications of Monoclonal anti-bodies.

-Monoclonal antibody use to treat breast cancer.

-We can add substance to anti-body

-

-f we have a cancer cells and at the surfaces of the cancer cells we can design anti-body that can bind specifically to antigen on the surface of the cancer cells, so we can target only cancer cells without kill normal cells.

-As we said we can add substance to ANTI-BODY TO USE IN DIFFERENT techniques such as western blooming, or ELISA.

Prefix	Target substem						Source substem (until 2017)		Stem		
	~1993	2009–2017	2017–2021	from 2021	meaning			meaning	old	from 2021	meaning
variable	—	-ami-	-ami-	-ami-	serum amyloid protein (SAP)/amyloidosis (pre-substem)		-a-	rat	-mab	-bart	artificial antibody
	-anibi-	—	—	—	angiogenesis (inhibitor)		-e-	hamster		-ment	fragment (derived from a variable domain)
	-ba(c)-	-b(a)-	-ba-	-ba-	bacterial		-i-	primate		-mig	multi-immunoglobulin (e.g. BsMAb)
	-ci(r)-	-c(i)-	-ci-	-ci-	cardiovascular		-o-	mouse		-tug	unmodified immunoglobulin
	-d(e)-	-d(e)-[a]	-de-[a]	-de-	endocrine		-u-	human			
	—	—	-eni-[b]	-eni-	enzyme inhibition		-xi-	chimeric (human/foreign)			
	-fung-	-f(u)-	-fung-	-fung-	fungal		-zu-	humanized			
	-gr(o)-	-gros-	-gros-	-gro-[c]	skeletal muscle mass related growth factors and receptors (pre-substem)		-xizu-	chimeric/humanized hybrid			
	-ki(n)-	-k(i)-	-ki-	-ki-	formerly: interleukin; from 2020: cytokine and cytokine receptor		-axo-	rat/mouse hybrid (see *trifunctional antibody*)			
	-les-	—	—	—	inflammatory lesions[7]						
	-li(m)-	-l(i)-	-li-	-ler-	immunomodulating	allergen	-vet-	veterinary use (pre-substem)[d]			
				-pru-		immunosuppressive					
				-sto-		immunostimulatory					
	-mul-	—	—	—	musculoskeletal system[8]						
	-ne(u)(r)-	-n(e)-	-ne-	-ne-	neural (nervous system)						
	-os-	-s(o)-	-os-	-os-	bone						
	-co(l)-	-t(u)-	-ta-	-ta-	colonic tumor	tumor					
	-go(t)-				testicular tumor						
	-go(v)-				ovarian tumor						
	-ma(r)-				mammary tumor						
	-me(l)-				melanoma						
	-pr(o)-				prostate tumor						
	-tu(m)-				miscellaneous tumor						
	-toxa-	-tox(a)-	-toxa-	-toxa-	toxin						
	—	—	-vet-	-vet-	veterinary use (pre-stem)[d]						
	-vi(r)-	-v(i)-	-vi-	-vi-	viral						

-

-The substance could be enzyme.

-Chemo Therapy is a traditional treatment use to treat cancer Disease but the Chemo therapy is very harmful, because it cant distinguish between Cancer cells and normal cells.

-Some Antibodies bind T cells with cancer cells

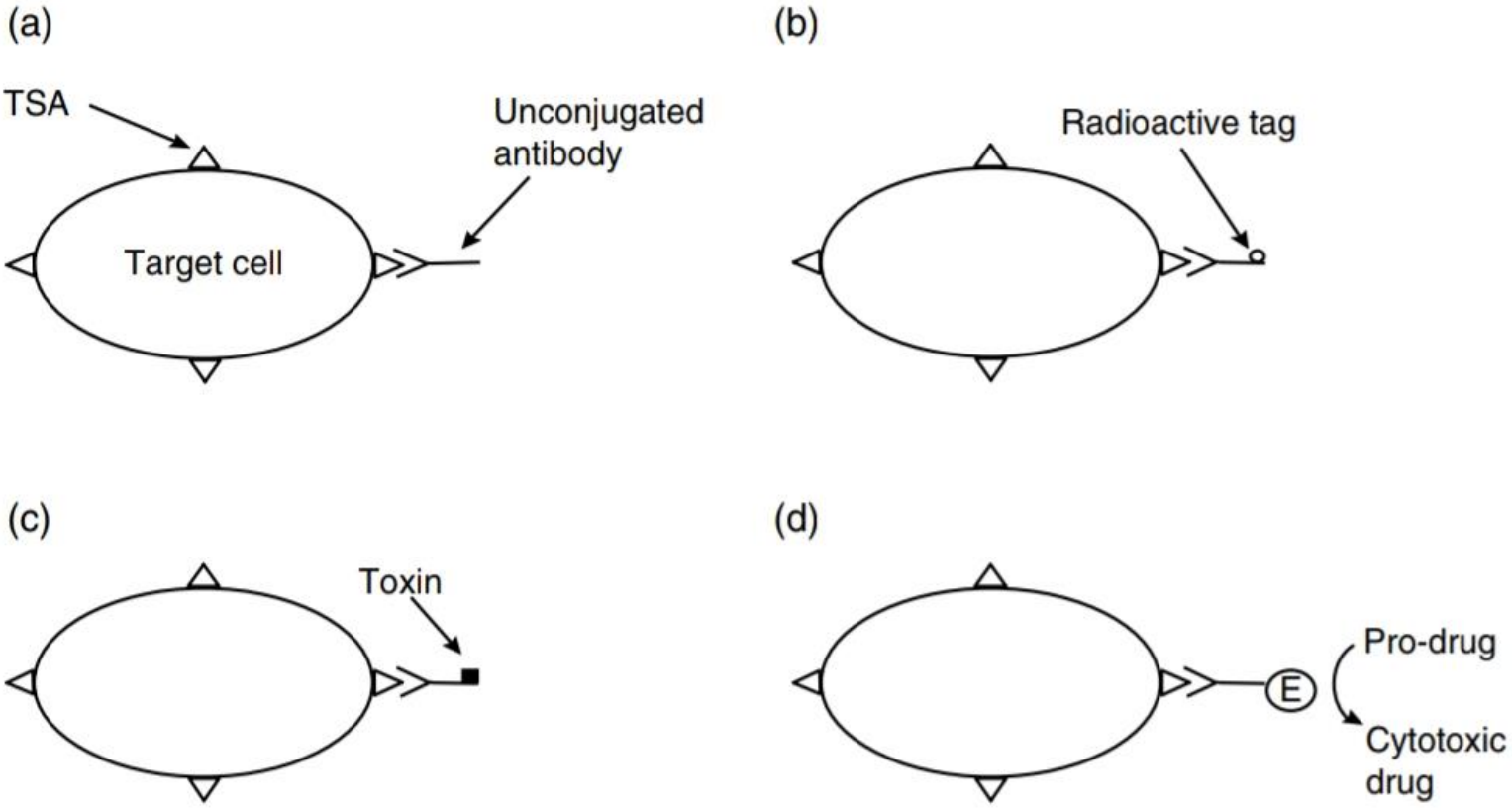
-T CELLS : on of the immune system cells that kill cancer cells.

-Immune lioposome : on there surface have antibodies and inside there have drugs

-The antibodies will allow to go to target cells

-

-The drugs will kill the target cells.



-Cytokine :protein that can tell immune system cells to clone and start attack the foreign molecules.

-Some time we use fragment of antibodies.

-Fragment antibodies produce be taken the Antibody and incubate with protease or by DNA Recombinant technology.

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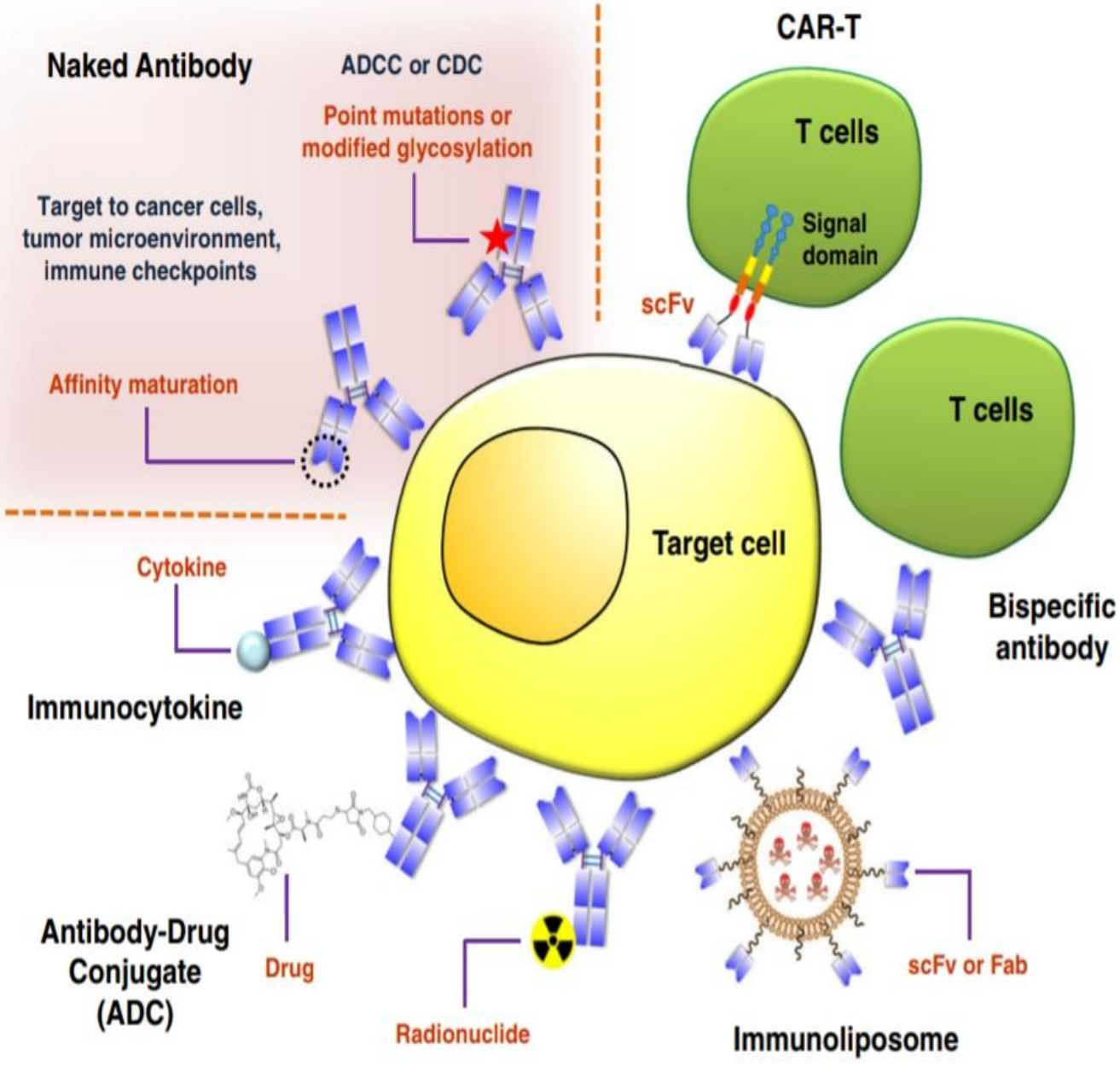

-Antibody divide in tow fragment

- Fab region
- Fac region

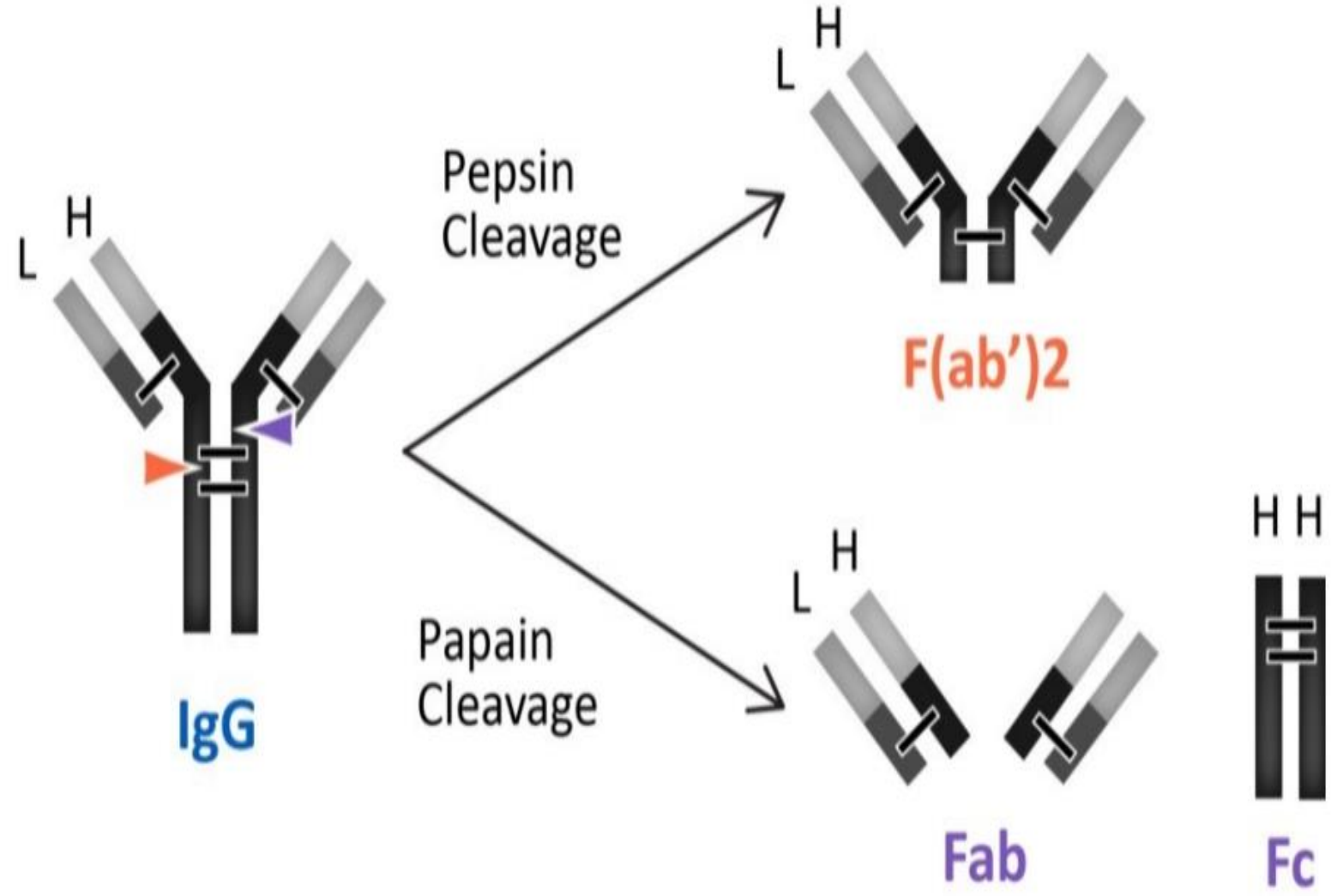
H
L
IgG
Pepsin
Cleavage
H
L
F(ab')2
Papain
Cleavage
H
L
Fab
H H
Fc

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Table 7.7 Antibody fragments approved for general medical use. For various commercial and technical reasons many of these products no longer remain on the market. Refer to text for details.

Product	Company	Indication	Approved
CEA-scan (arcitumomab). Murine Mab fragment (Fab) directed against human carcinoembryonic antigen (CEA). Produced in a hybridoma cell line	Immunomedics Inc.	Detection of recurrent/metastatic colorectal cancer	1996 (USA and EU) Withdrawn 2005
Cimzia (certolizumab pegol). Anti-TNF-α humanized antibody Fab′ fragment, PEGylated. Produced in *E. coli*	UCB	Crohn's disease, rheumatoid arthritis	2009 (EU) 2008 (USA)
Indimacis 125 (igovomab). Murine Mab fragment (Fab_2) directed against the tumour-associated antigen CA125. Produced in a hybridoma cell line	Cisbio International	Diagnosis of ovarian adenocarcimona	1996 (EU) Withdrawn 2009
LeukoScan (sulesomab). Murine Mab fragment (Fab) directed against NCA-90, a surface granulocyte non-specific cross-reacting antigen. Produced in a Sp2/0 cell line	Immunomedics GmbH	Diagnostic imaging for infection/ inflammation in bone of patients with osteomyelitis	1997 (EU)
Lucentis (ranibizumab). Humanized Mab fragment. Binds and inactivates VEGF-A. Produced in *E. coli*	Novartis, Genentech	Neovascular (wet) age-related macular degeneration	2007 (EU) 2006 (USA)
MyoScint (imiciromab pentetate). Murine Mab fragment directed against human cardiac myosin. Produced in a hybridoma cell line	Janssen	Myocardial infarction imaging agent	1996 (USA) Withdrawn 1999
ReoPro (abciximab). Fab fragments derived from a chimeric Mab directed against the platelet surface receptor GPIIb/IIIa. Produced in a mammalian cell line	Eli Lilly	Prevention of blood clots	1994 (USA)
Tecnemab K1 (anti-melanoma Mab fragments). Murine Mab fragments (Fab/Fab_2 mix) directed against high-molecular-weight melanoma-associated antigen. Produced in murine ascites culture	Sorin	Diagnosis of cutaneous melanoma lesions	1996 (EU) Withdrawn 2000
Verluma (nofetumomab). Murine Mab fragments (Fab) directed against carcinoma-associated antigen. Produced in murine hybridoma cell line	NeoRx	Detection of small cell lung cancer	1996 (USA) Withdrawn 1999

Mab, monoclonal antibody; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

-Fab region :allow antibody to bind to antigen.

-Fac :allow antibody to stay in blood couple of Days.

We can produce Antibody fragment by use enzyme called pepsin, it's a protease.

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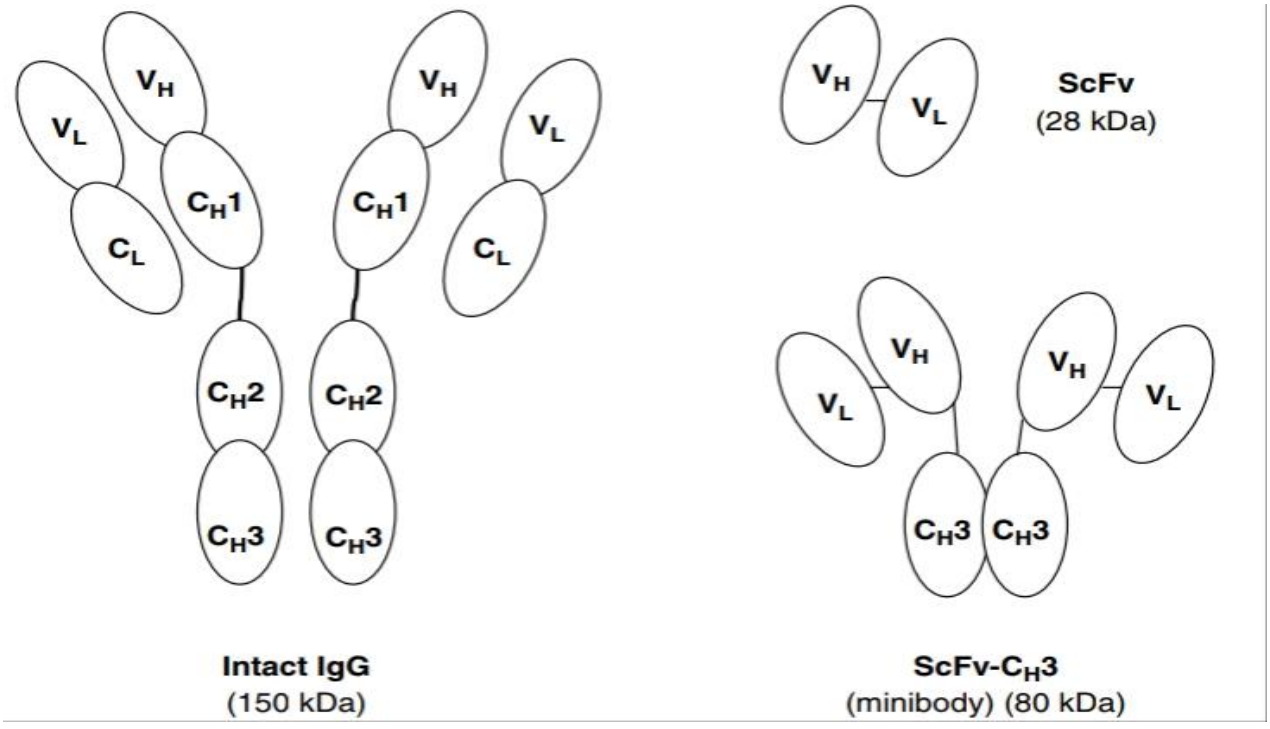

-We need small Antibody, because the big Antibody cant go inside tumor tissues and bind all cells inside it.

-Mini body : has variable region and only constant region.

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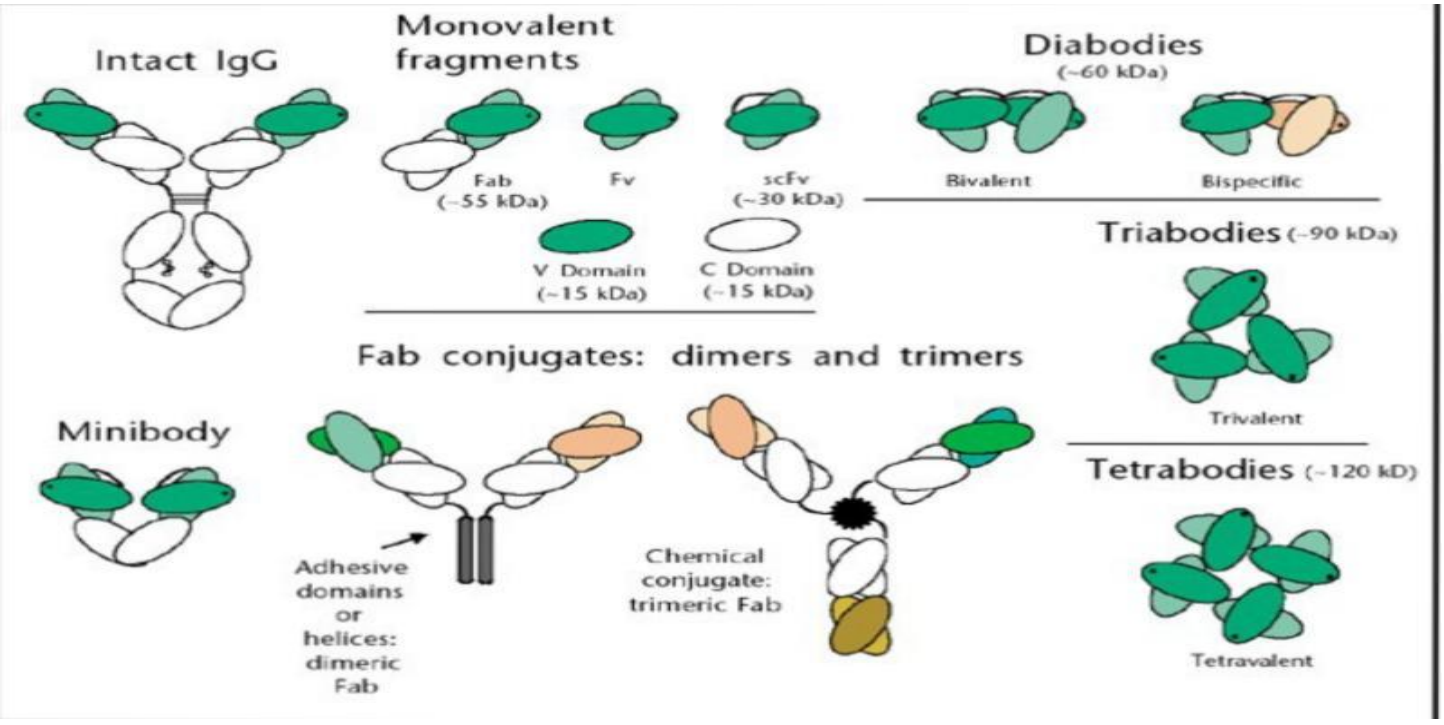
Intact IgG
Monovalent fragments
Fab (~55 kDa)
Fv
scFv (~30 kDa)
V Domain (~15 kDa)
C Domain (~15 kDa)
Diabodies (~60 kDa)
Bivalent
Bispecific
Triabodies (~90 kDa)
Trivalent
Tetrabodies (~120 kD)
Tetravalent
Fab conjugates: dimers and trimers
Minibody
Adhesive domains or helices: dimeric Fab
Chemical conjugate: trimeric Fab

-

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