Monoclonal Antibodies (II)

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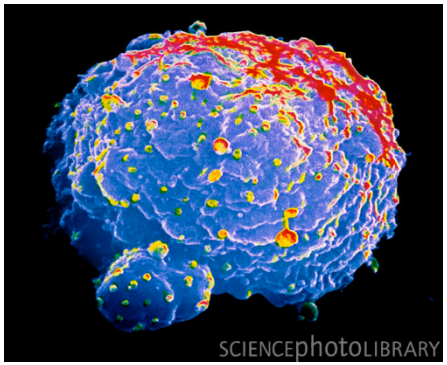
Objectives of this lecture

By the end of this lecture you will be able to:

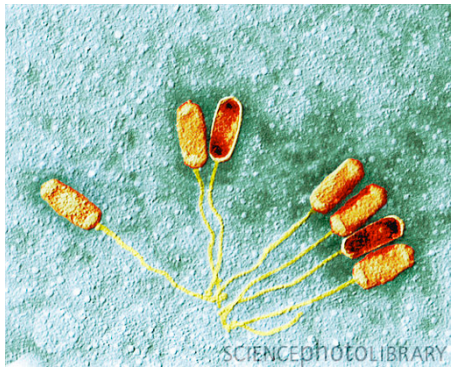
1. Define terms such as monoclonal, polyclonal, isotype, idiotype, allotype, CDR, and hybridoma
2. Compare monoclonal-antibody production methods
3. Identify different mAb types
4. List some applications of mAb in medicine

mAb Production

Hybridoma Technology

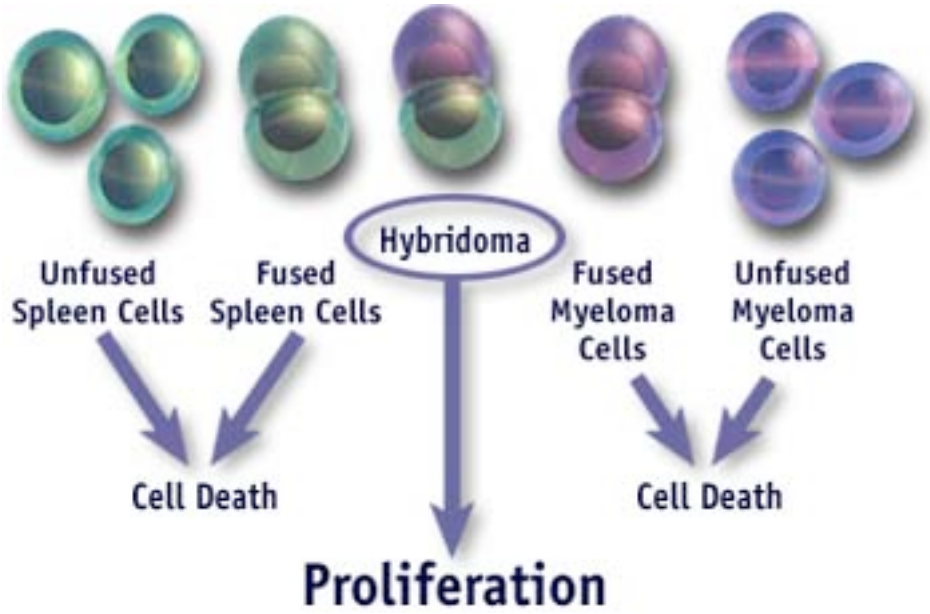


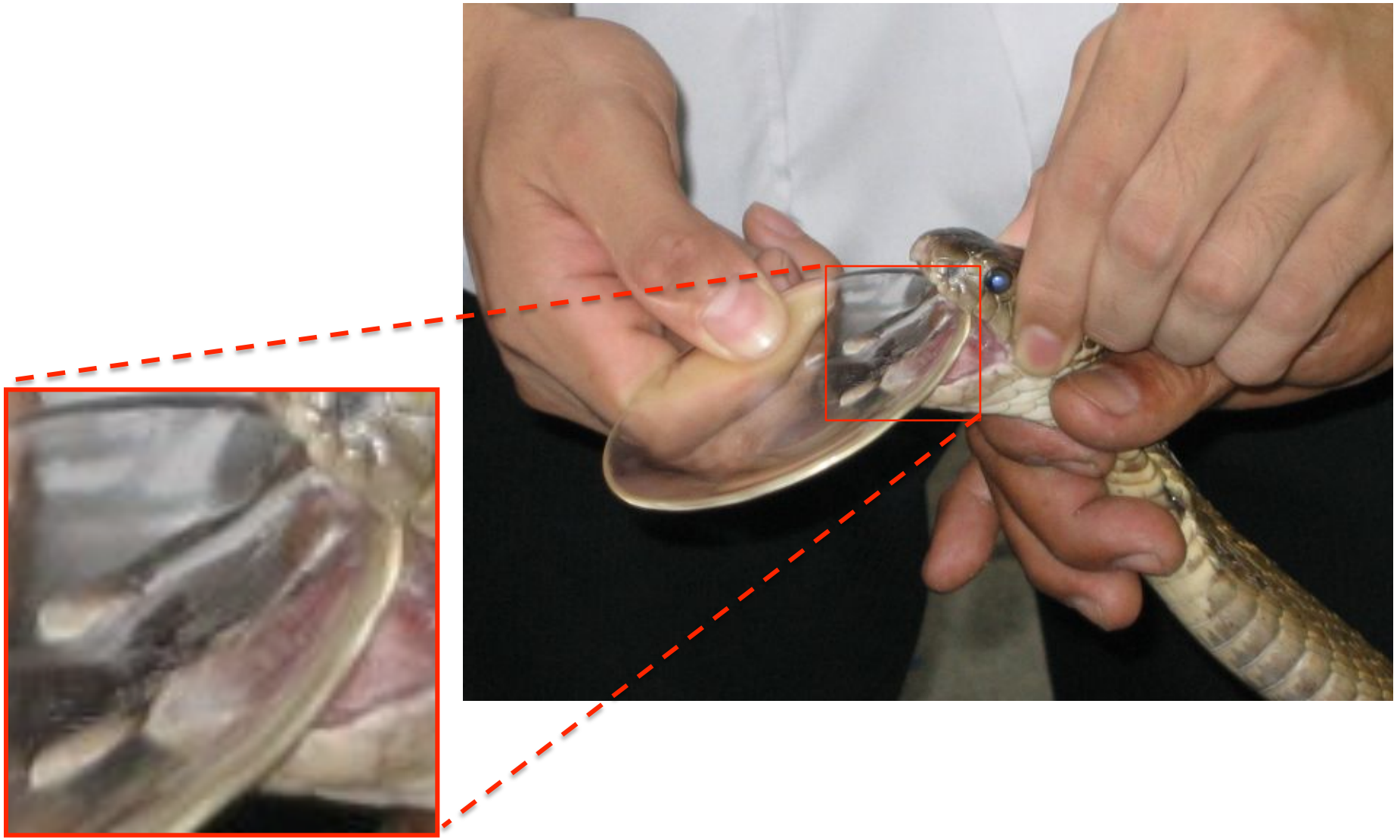
Phage Display Method

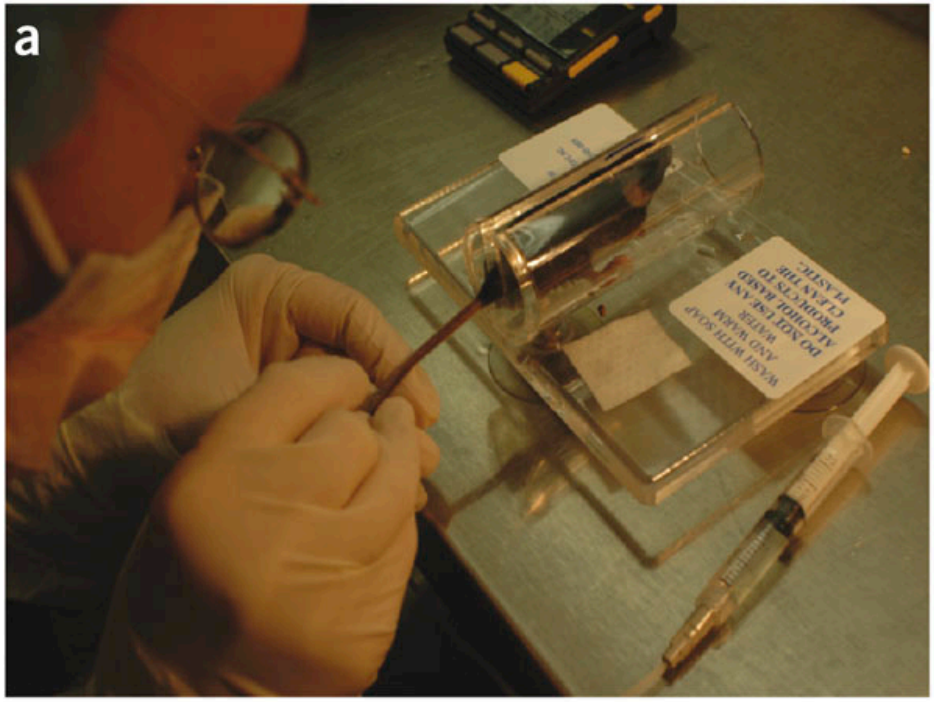


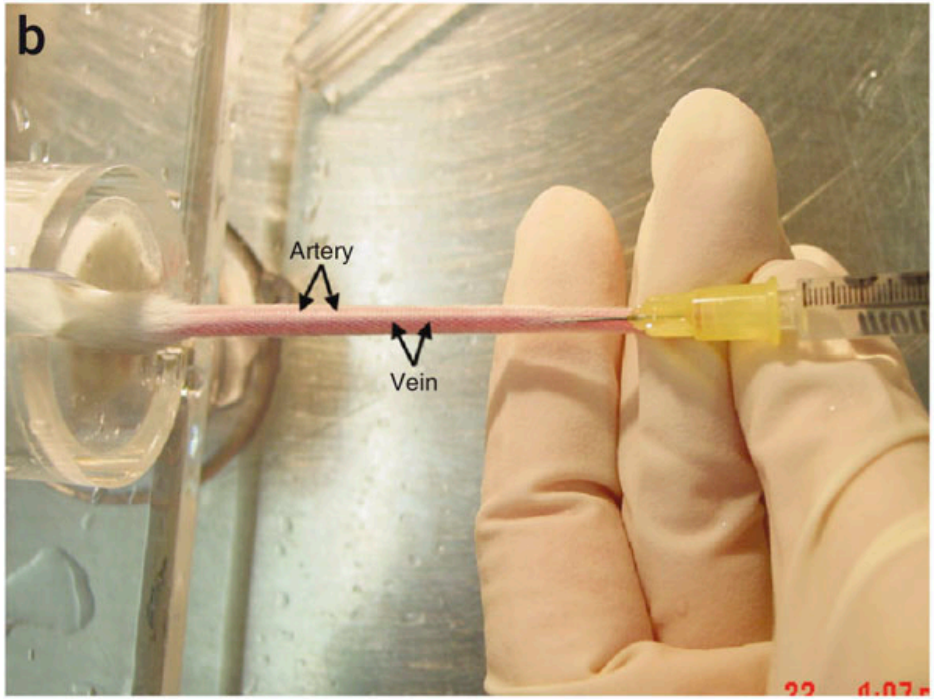
Hybridoma Technology

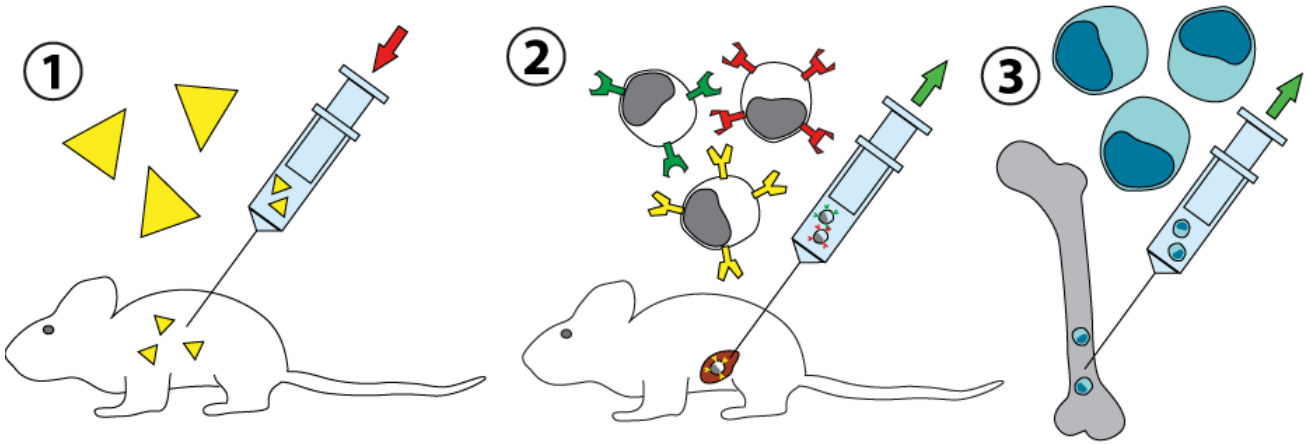
1975, by Georges Köhler and Cesar Milstein
- Be awarded a Nobel Prize in1984



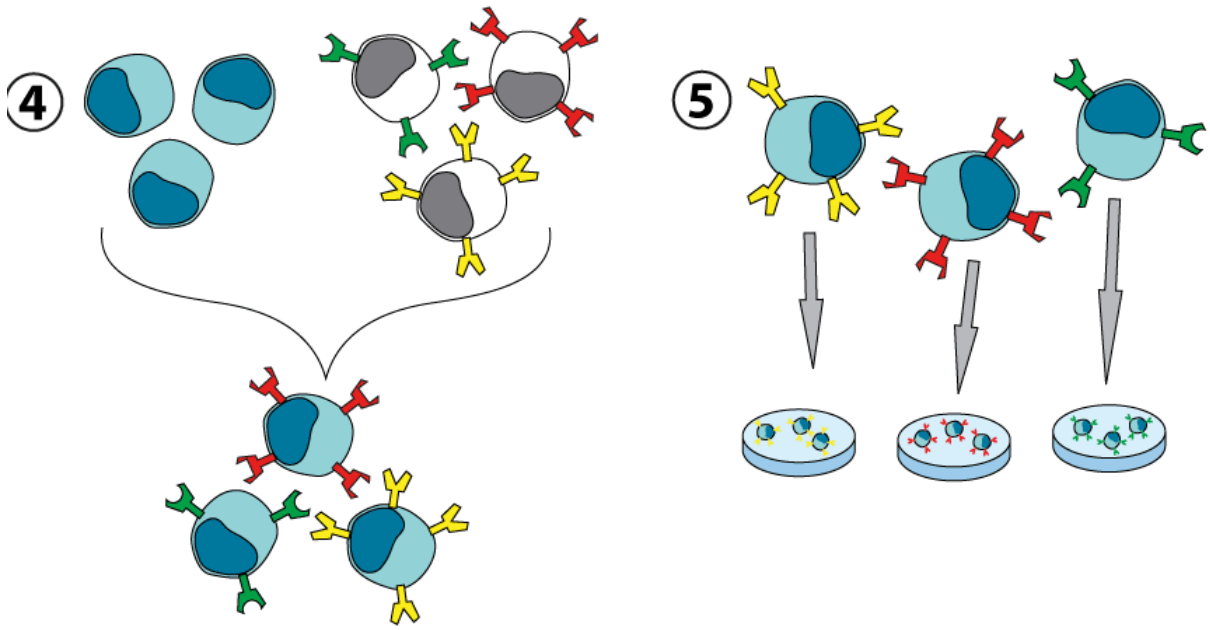



a

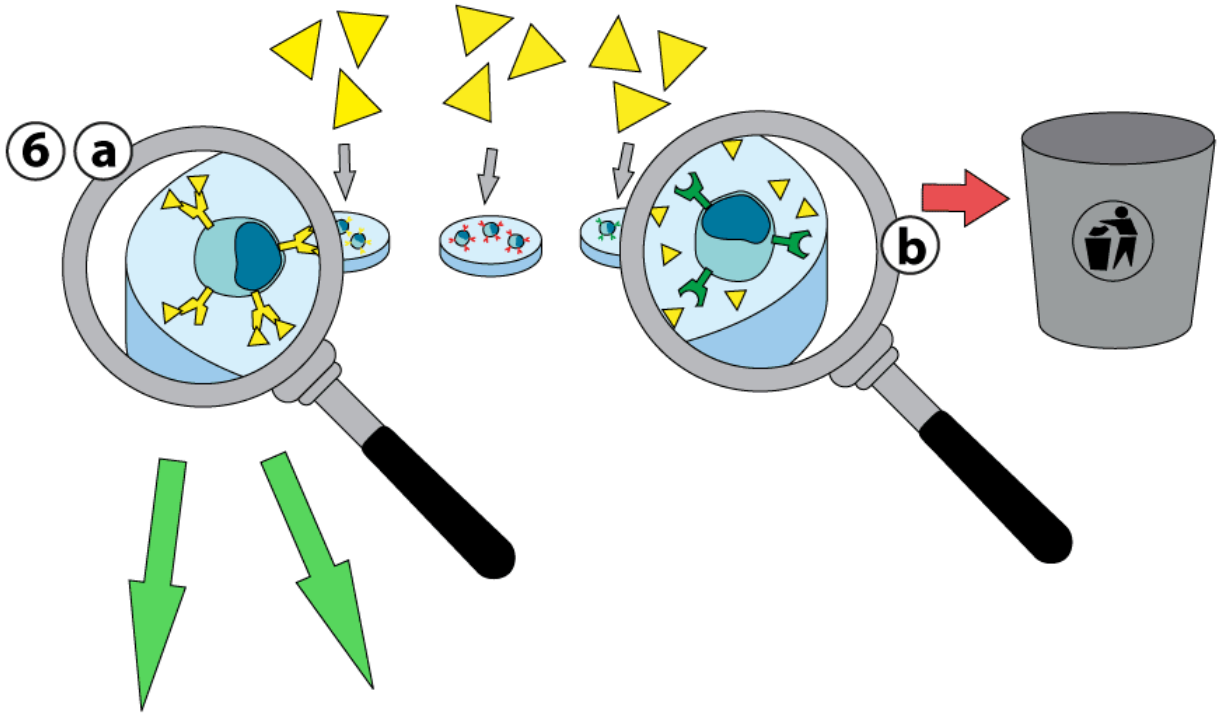

b
Artery
Vein



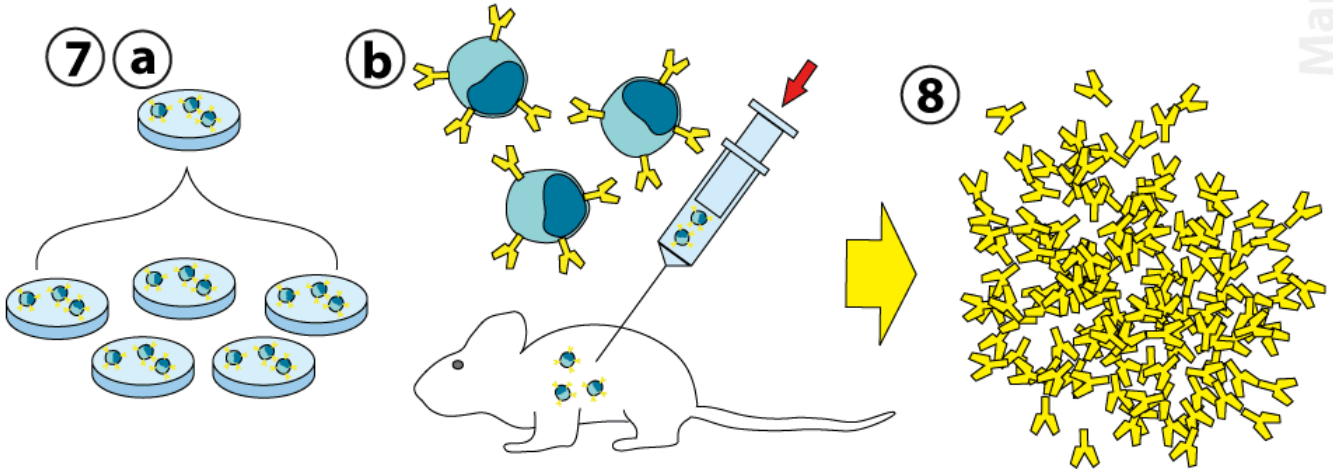
(1) Immunisation of a mouse
(2) Isolation of B cells from the spleen
(3) Cultivation of myeloma cells



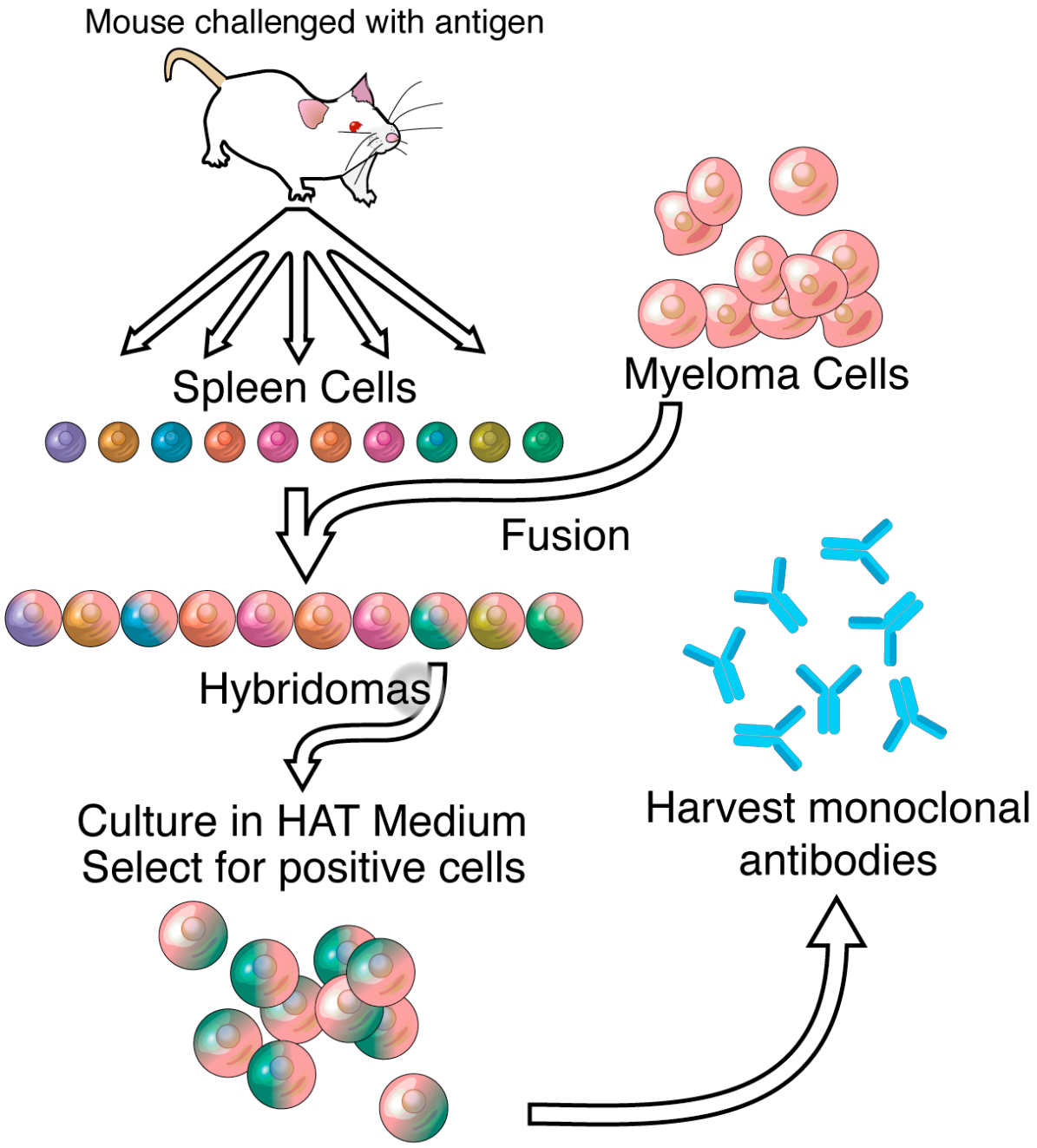
(4) Fusion of myeloma and B cells (using PEG)
(5) Separation of cell lines



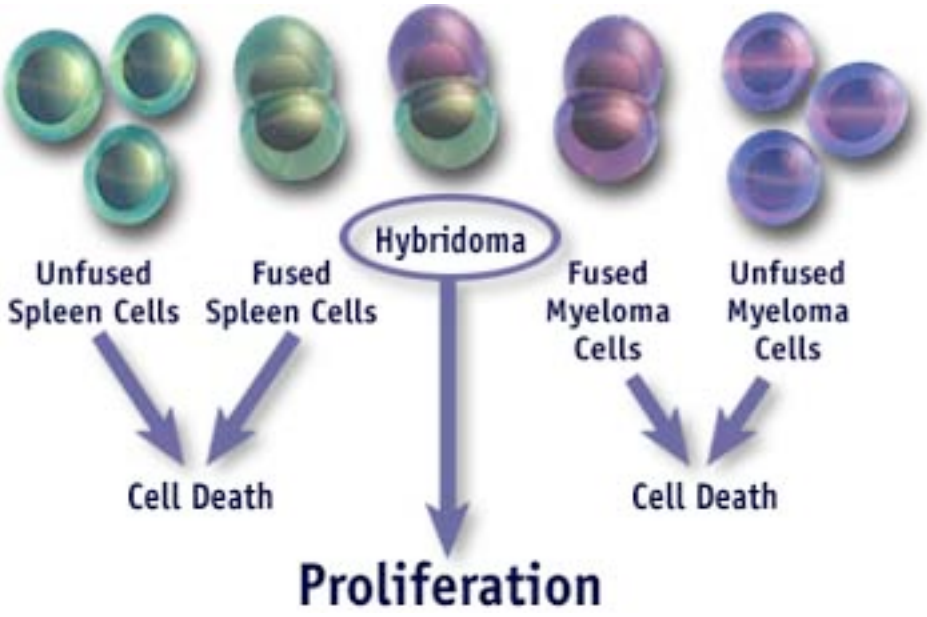
(6) Screening of suitable cell lines



(7) *in vitro* **(a)** or *in vivo* **(b)** multiplication
(8) Harvesting


Mouse challenged with antigen
Spleen Cells
Myeloma Cells
Fusion
Hybridomas
Culture in HAT Medium
Select for positive cells
Harvest monoclonal antibodies

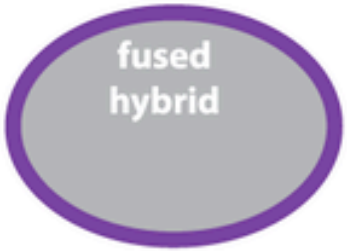
HAT Selection



Selected by using HAT medium (**H**ypoxanthine-**A**minopterin-**T**hymidine)

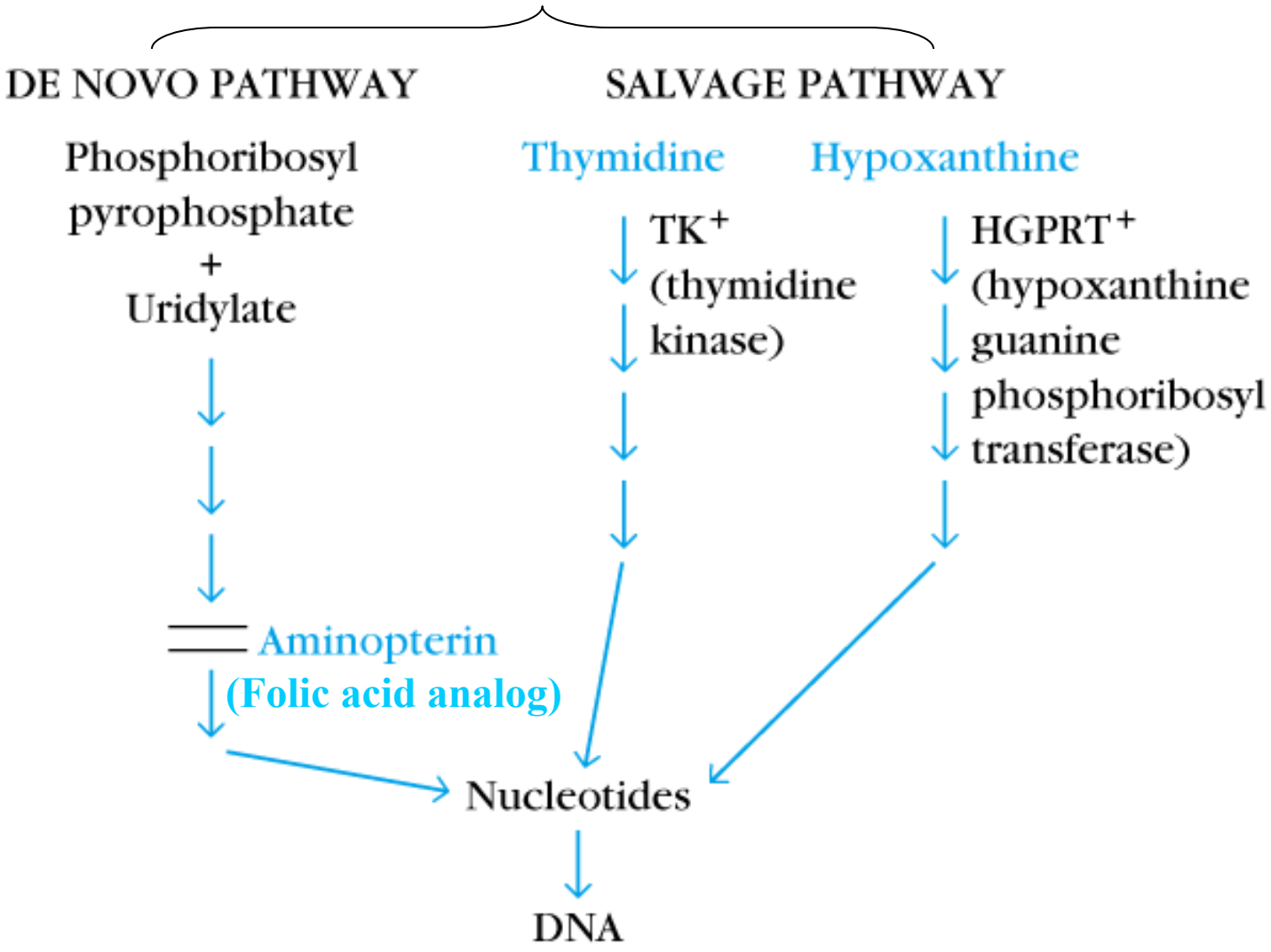
- Myeloma cells are unable to grow
- B cells are able to survive, but can not live for extended periods

HAT Selection

	TK -	TK+/TK -	TK +
Genotype:*			
Cell type:			
HAT fate:	**DIES**	**SURVIVES**	**DIES**
Explanation:	**Unable to synthesize DNA:** (1) Thymidine kinase* mutation causes a loss-of-function in the "salvage" pathway and (2) Aminopterin blocks "De novo" pathway.	**Immortal and restored DNA synthesis:** (1) Immortality from plasmacytoma and (2) rescued ability to synthesize DNA due to restored thymidine kinase* function.	**Mortal:** (1) Functional DNA synthesis, but (2) eventually dies because of limited number of replication cycles

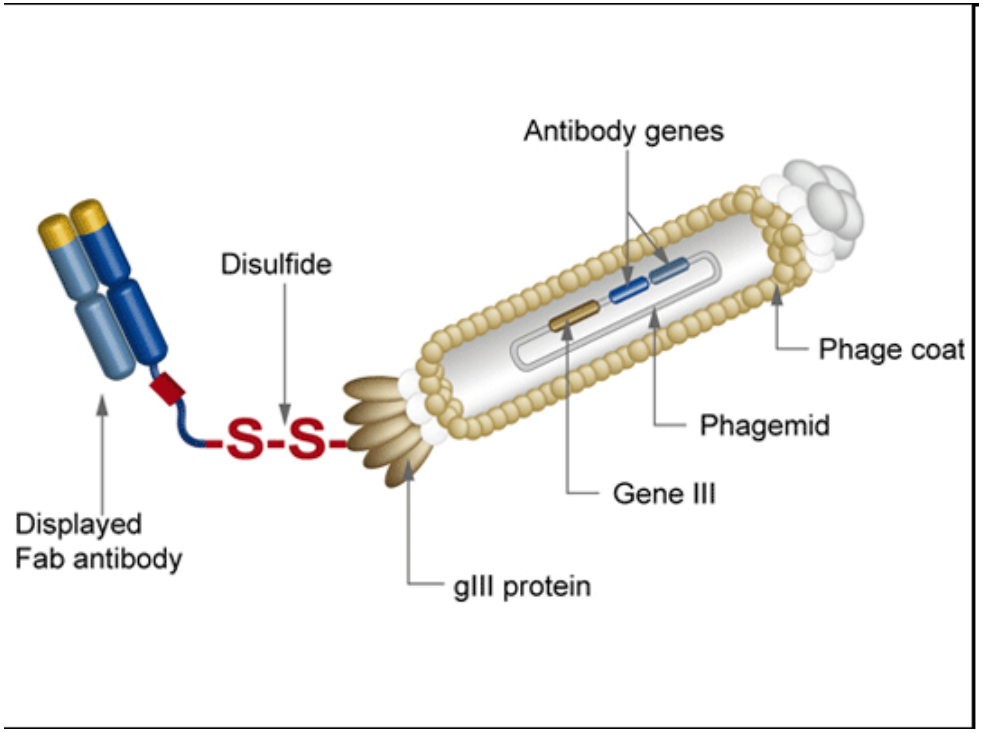
**HGPRT (hypoxanthine-guanine phosphoribosyltransferase) mutants can be used in place of TK (thymidine kinase) mutants*

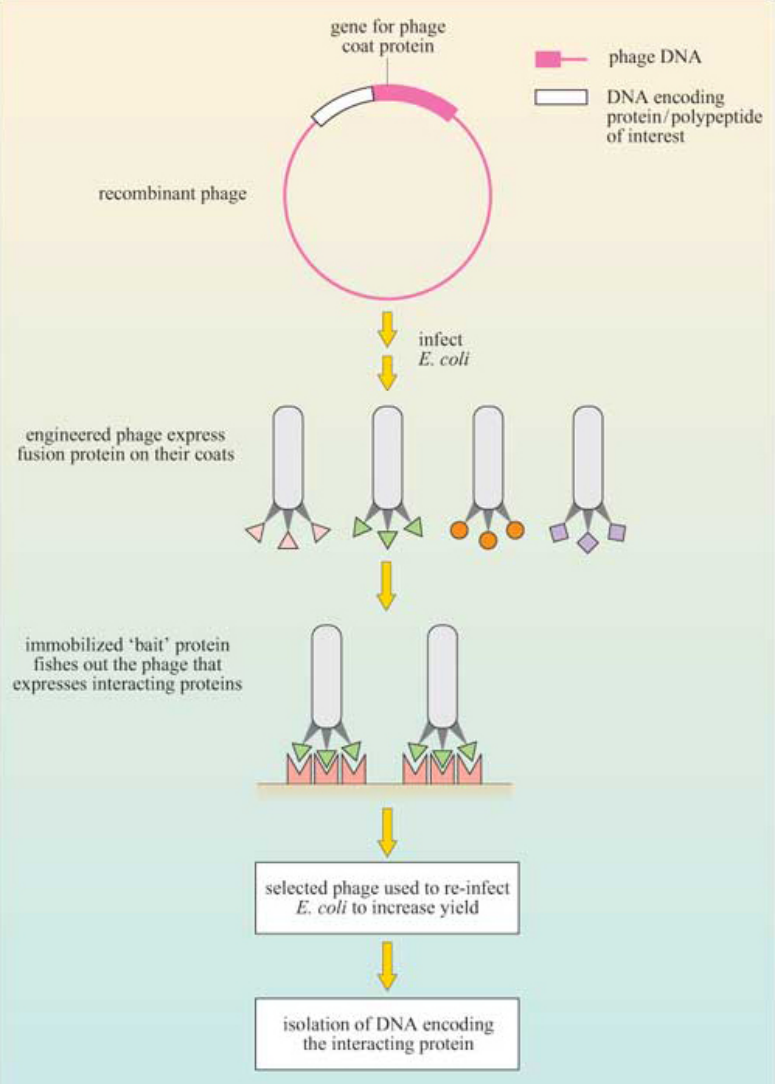
Two different pathways to synthesis nucleotide in mammalian cells

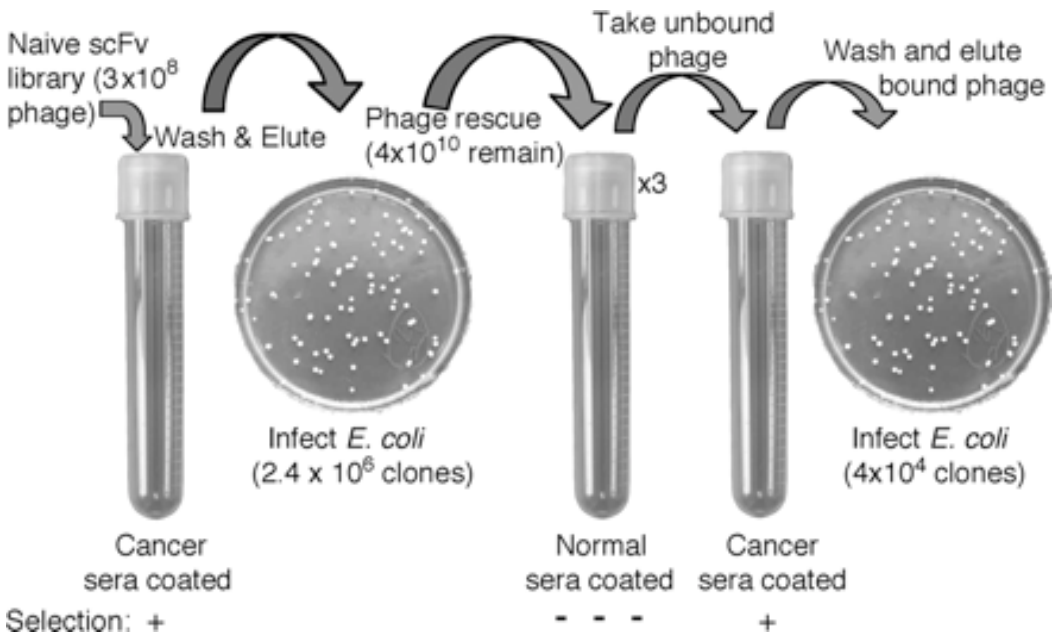


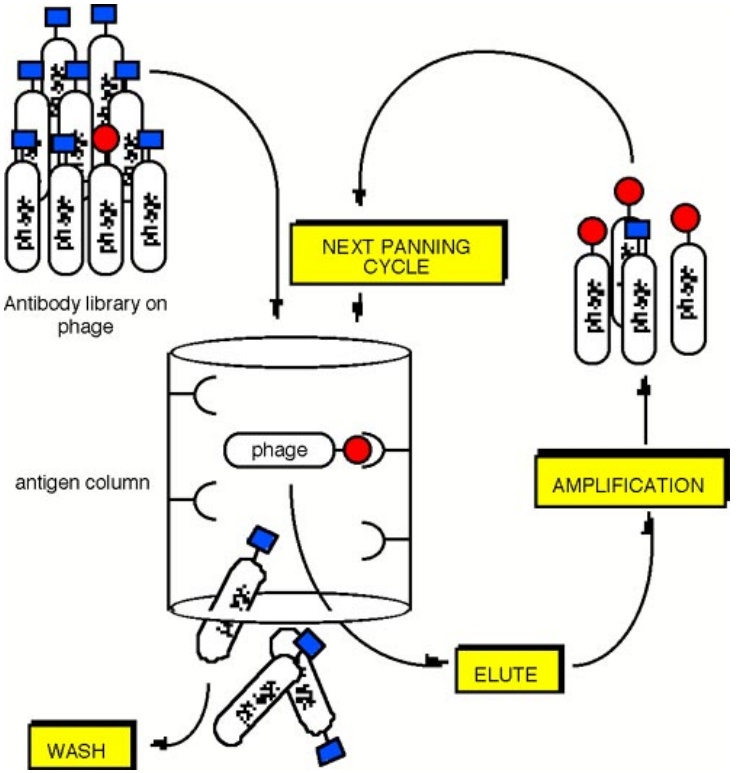
Phage Display - Introduction

- The display of functional foreign peptides or small proteins on the surface of bacteriophage particles.
- An important tool in protein engineering
- A powerful way to screen and select for peptides on the basis of binding or molecular recognition





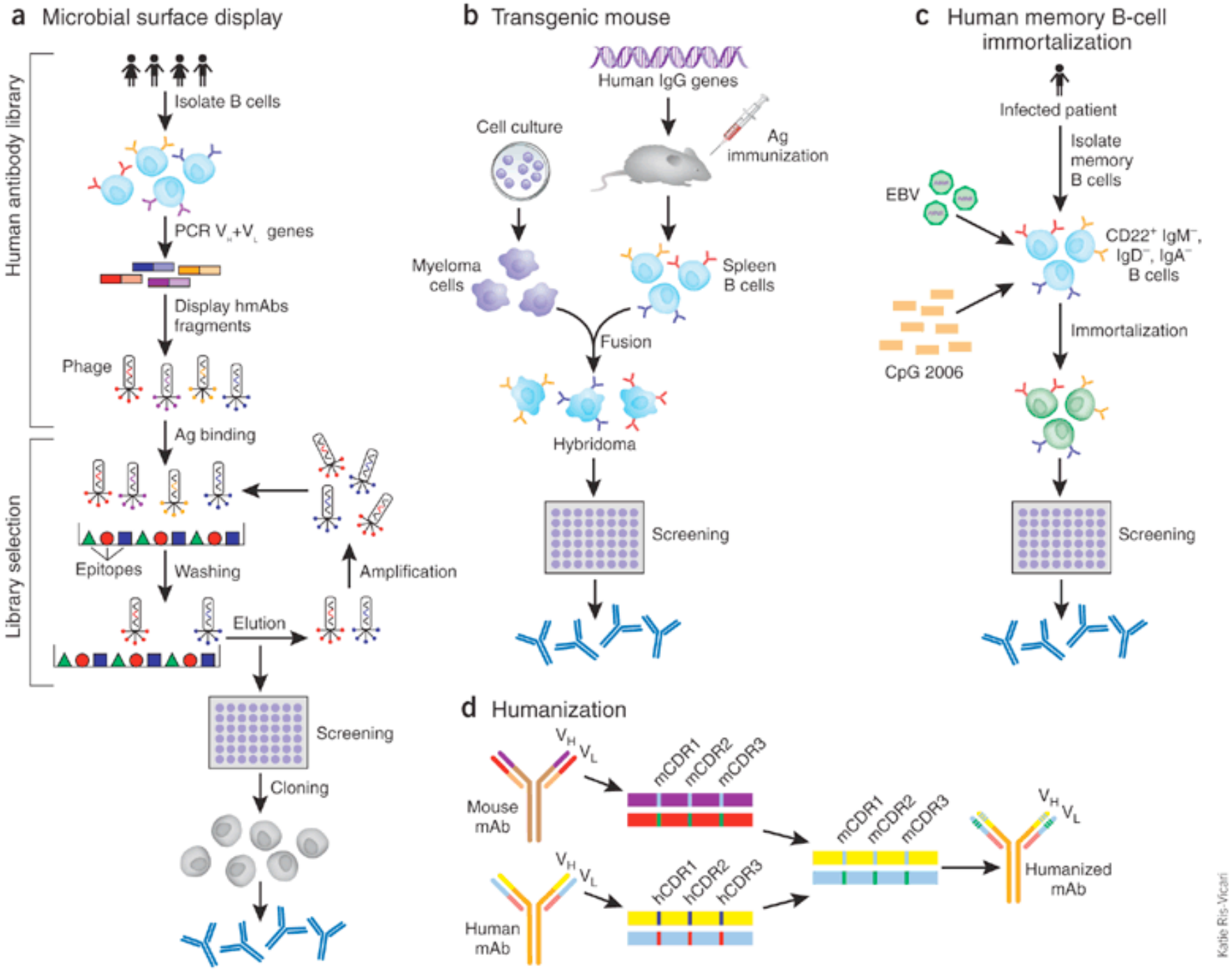




Phage Display Principle

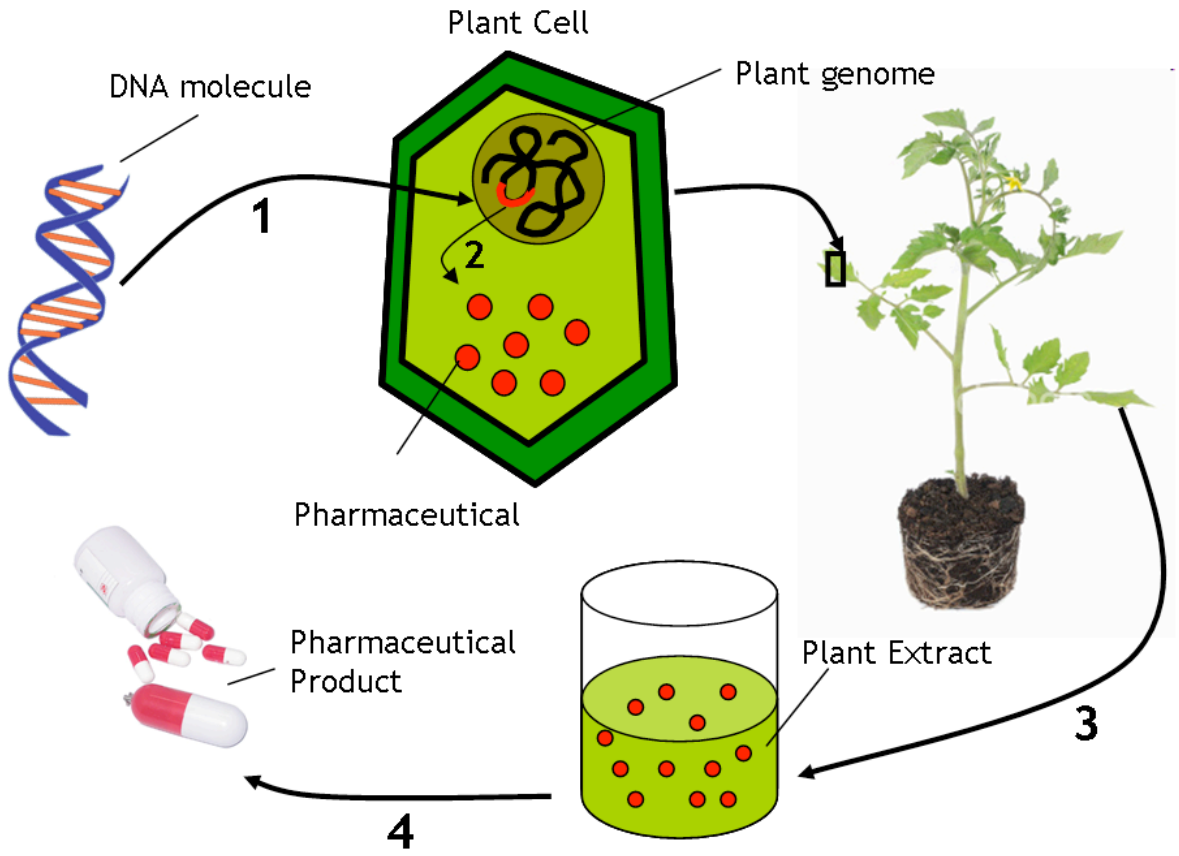
Phage Display Advantages

- More efficient than hybridoma system.
- Cheaper to produce recombinant antibodies using bacteria, rather than mammalian cell line.
- Easier to maintain and grow bacterial cultures for recombinant antibody production.
- Bypass immunization in antibody selection.
- Bypass the use of animal cells for production of antibodies.
- Producing the combinatorial library (ideally with 10^8 to 10^9 members) of functional antibodies to generate a larger repertoire of antibodies than those available through conventional hybridoma technology.


a Microbial surface display
Human antibody library
Isolate B cells
PCR V_H+V_L genes
Display hmAbs fragments
Phage
Ag binding
Library selection
Epitopes
Washing
Amplification
Elution
Screening
Cloning
b Transgenic mouse
Human IgG genes
Cell culture
Ag immunization
Myeloma cells
Spleen B cells
Fusion
Hybridoma
Screening
c Human memory B-cell immortalization
Infected patient
Isolate memory B cells
EBV
CD22+ IgM−, IgD−, IgA− B cells
CpG 2006
Immortalization
Screening
d Humanization
V_H V_L
Mouse mAb
mCDR1 mCDR2 mCDR3
Human mAb
hCDR1 hCDR2 hCDR3
Humanized mAb
Katie Ris Vicari

Plantibodies

- "**plantibodies**" are antibodies produced by **genetically-engineered corps** e.g. corn, potatoes and tobacco plant
- "**plantibodies**" are cheaper and arguably safer than mammalian mAbs

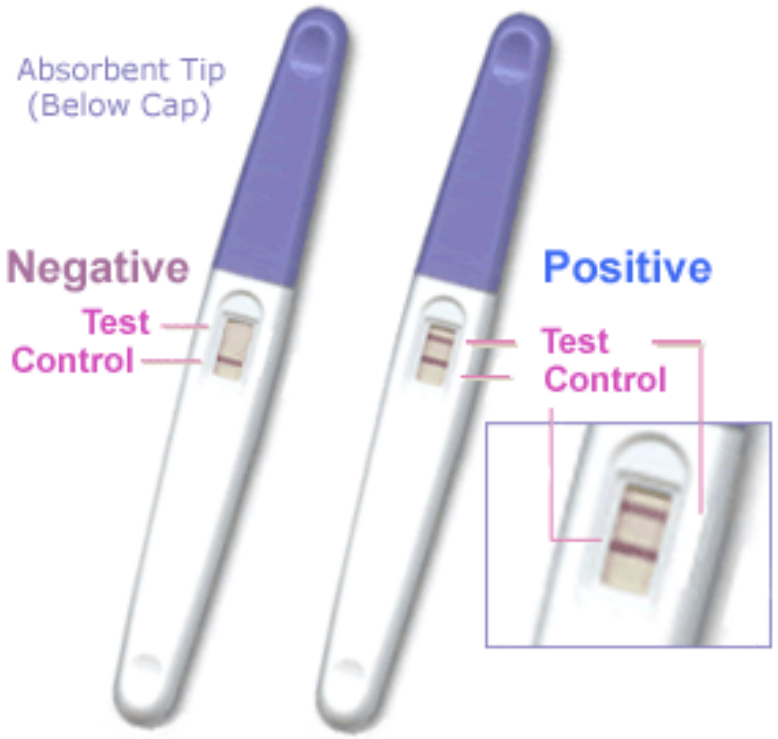


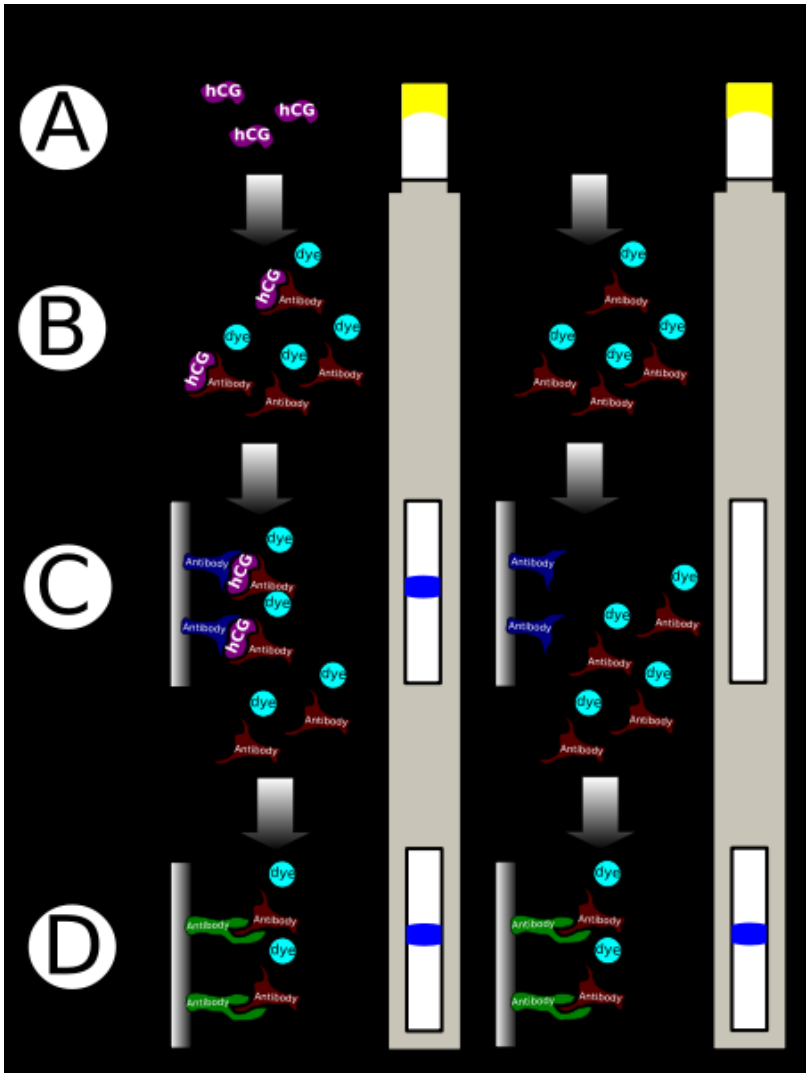
Clinical Applications of Monoclonal Antibodies

A. Diagnosis
   1. Pregnancy test
   2. ELISA
   3. Western Blot
   4. Flow Cytometry
   5. Radioimmunoimaging

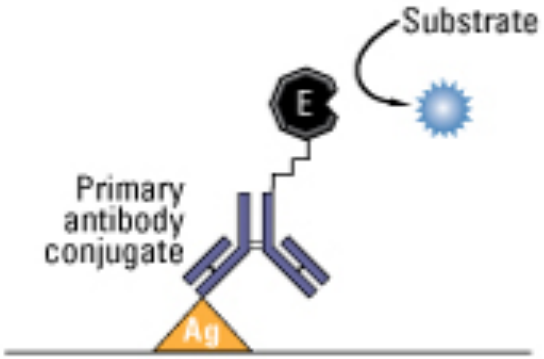
B. Therapy
   A. Passive Immunotherapy
   B. Active Immunotherapy
   C. Drug Targeting

Pregnancy test

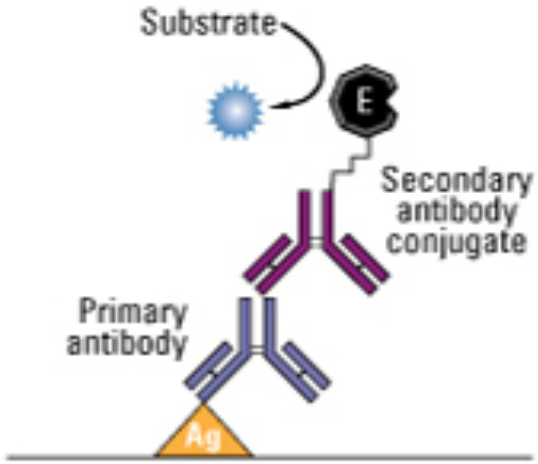




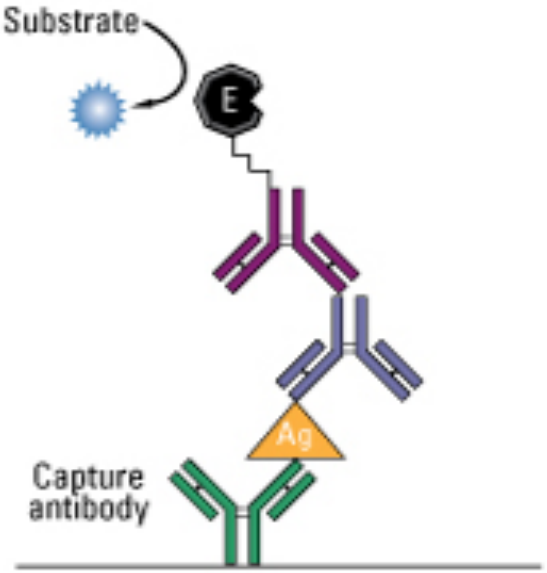
ELISA



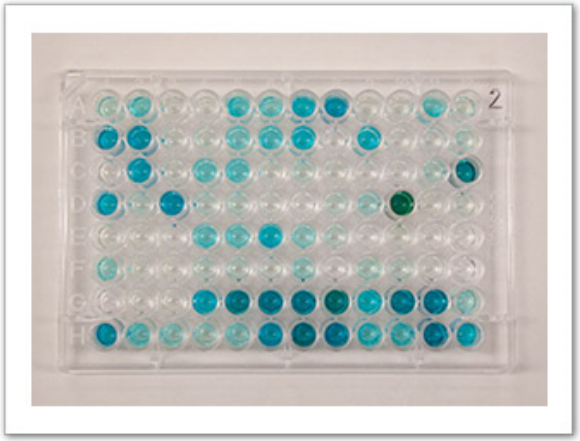
Direct Assay

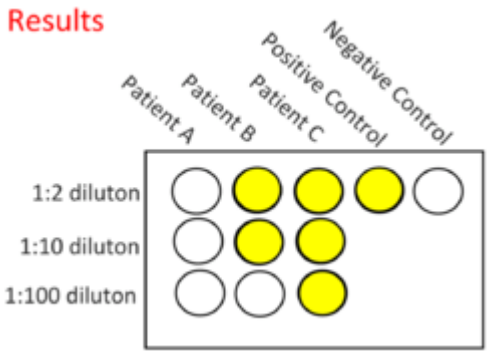


Indirect Assay

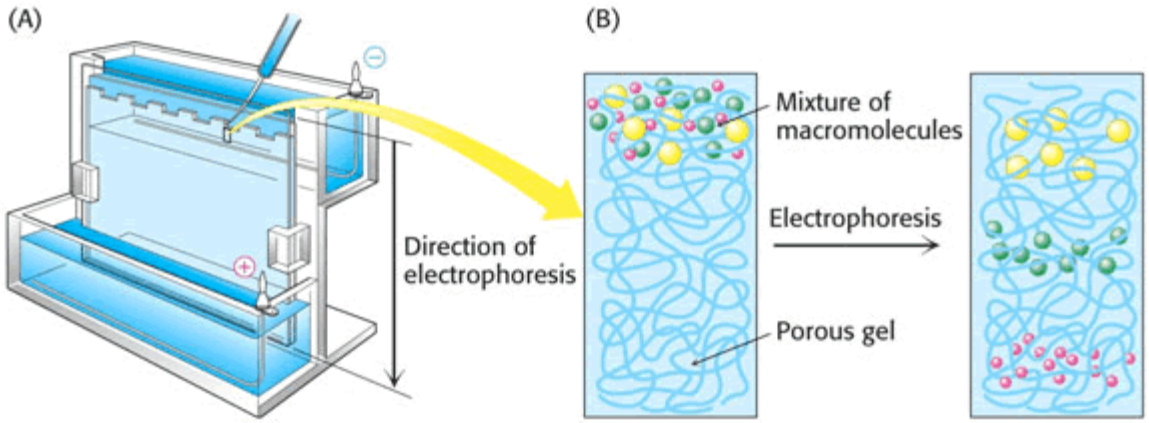


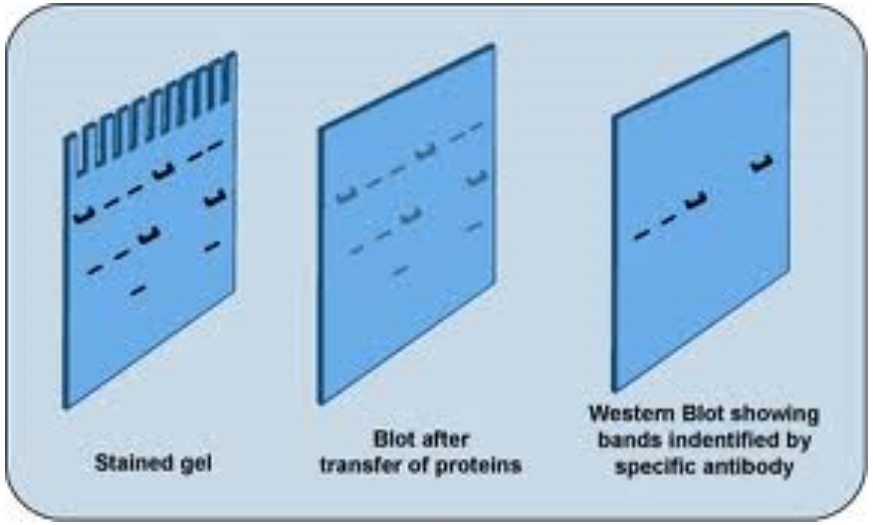
Capture Assay
"Sandwich"



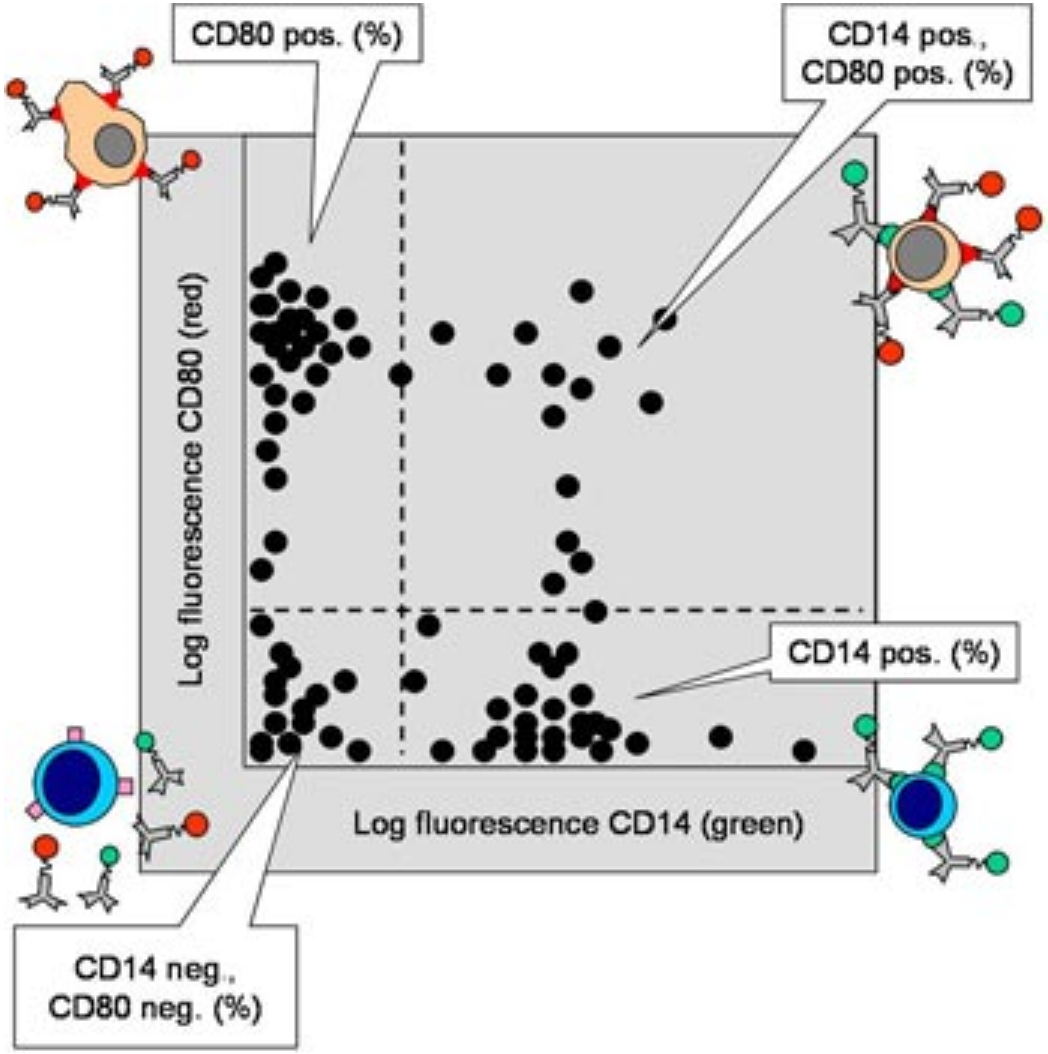


Western Blot

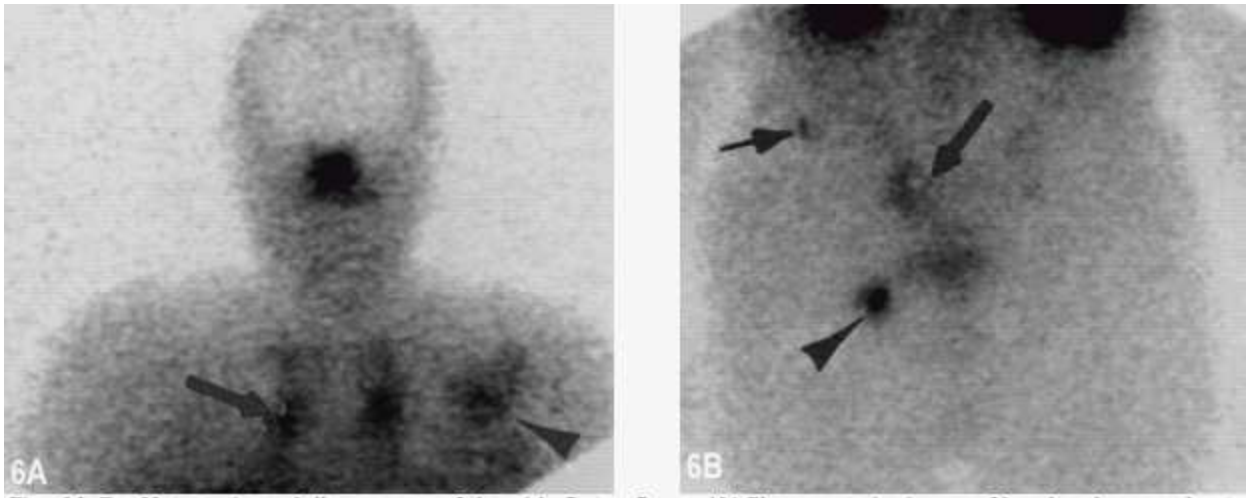




Flow cytometry

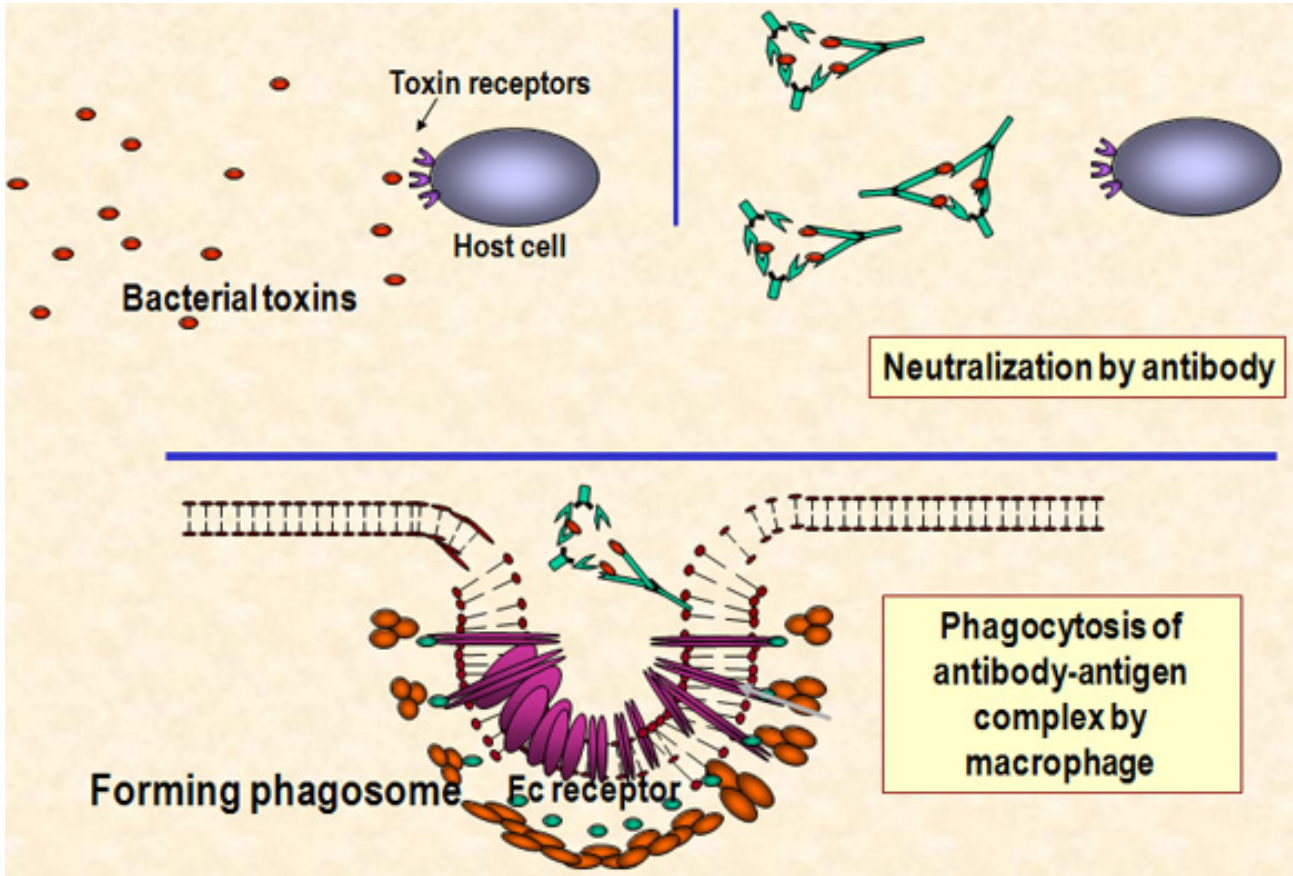


Radioimmunoimaging

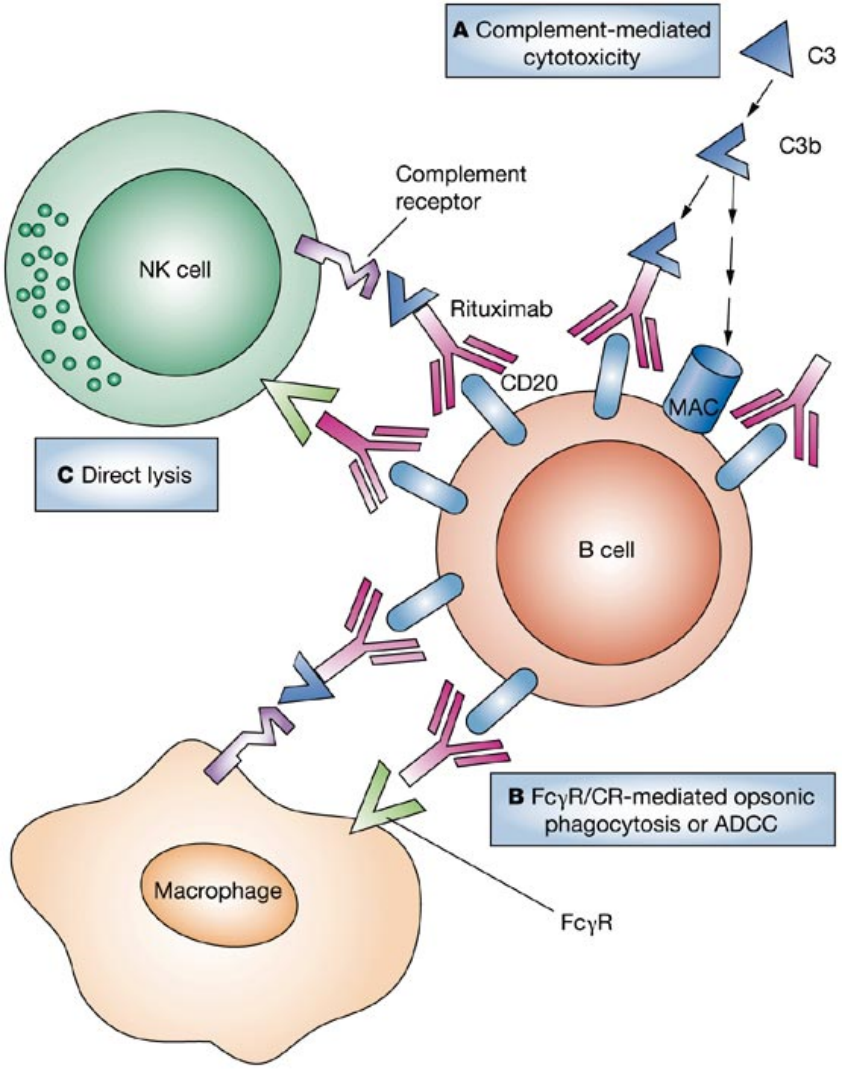


Figs 6A-B. - Metastatic medullary cancer of thyroid. OctreoScan. (A) Planar anterior image of head and upper chest. Metastatic lesions are evident in the mediastinum (arrow) and left axilla (arrowhead). (B) Planar scan of posterior pelvis view shows metastatic lesions in the sacrum (arrow), left pubic symphysis (arrowhead), and left iliac crest (small arrow).

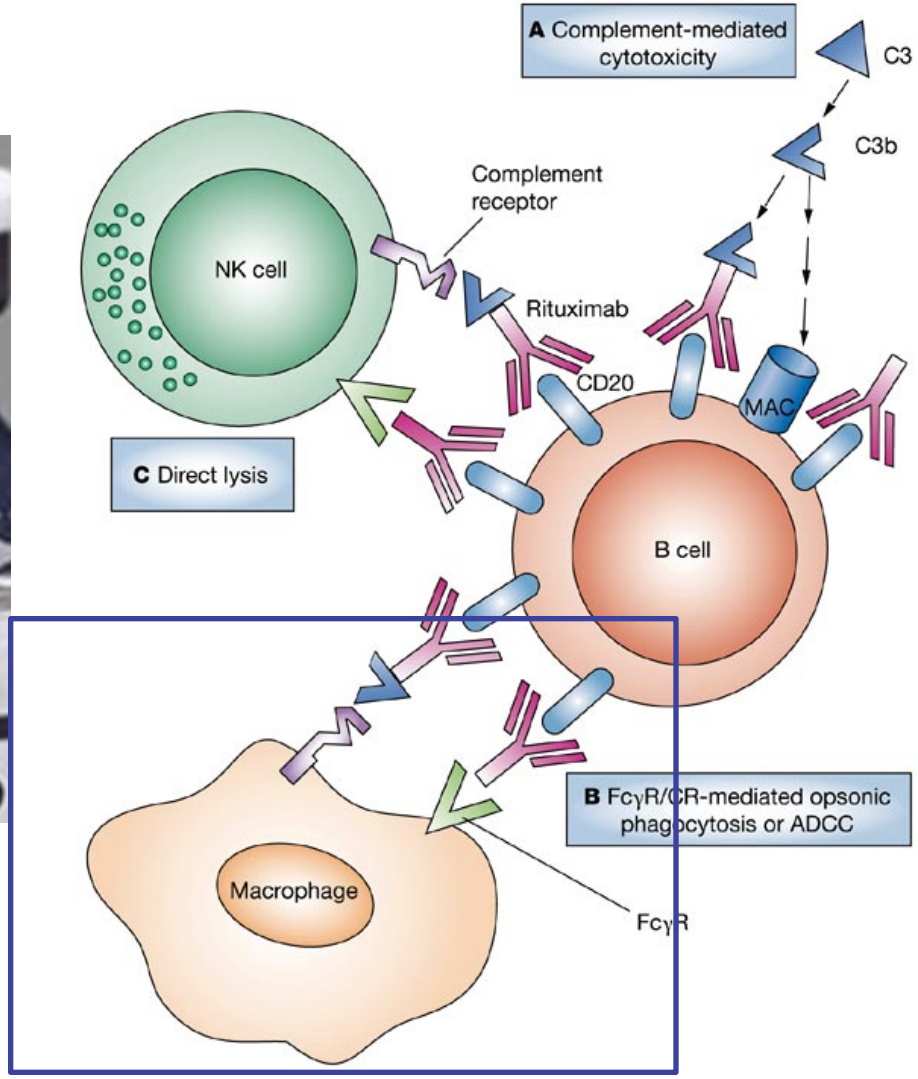
Passive immunotherapy



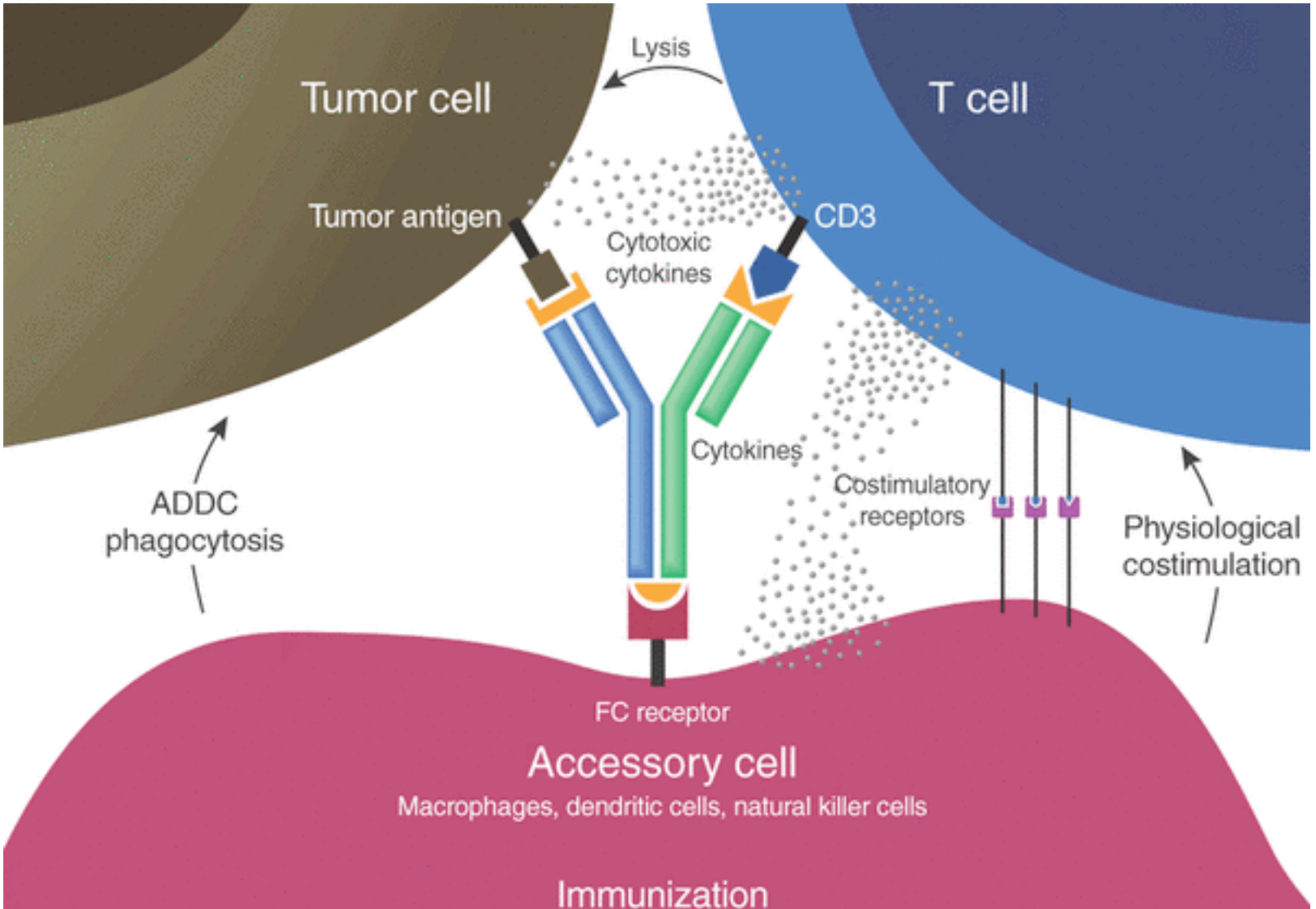
Passive immunotherapy



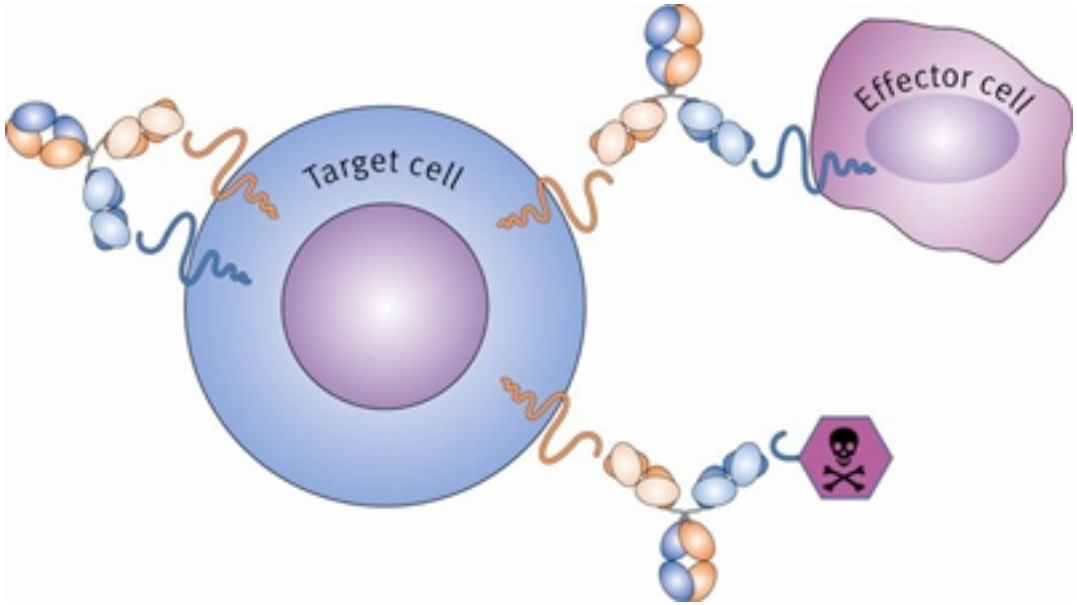
Active immunotherapy



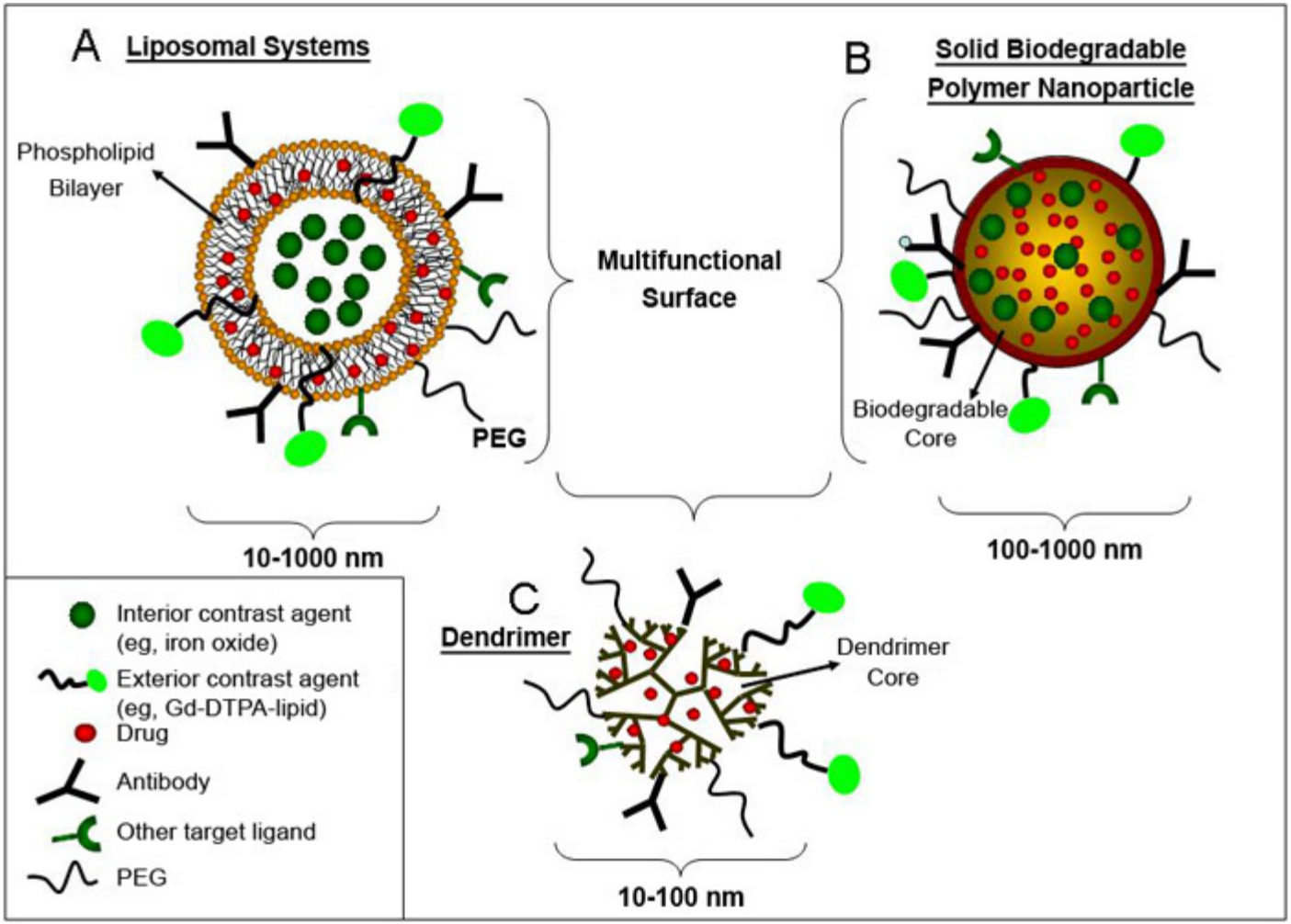
Active immunotherapy using Bispecific mAb



Bispecific mAb



Drug targeting



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- ✓ Compare monoclonal-antibody production methods
- ✓ Identify different mAb types
- ✓ List some applications of mAb in medicine