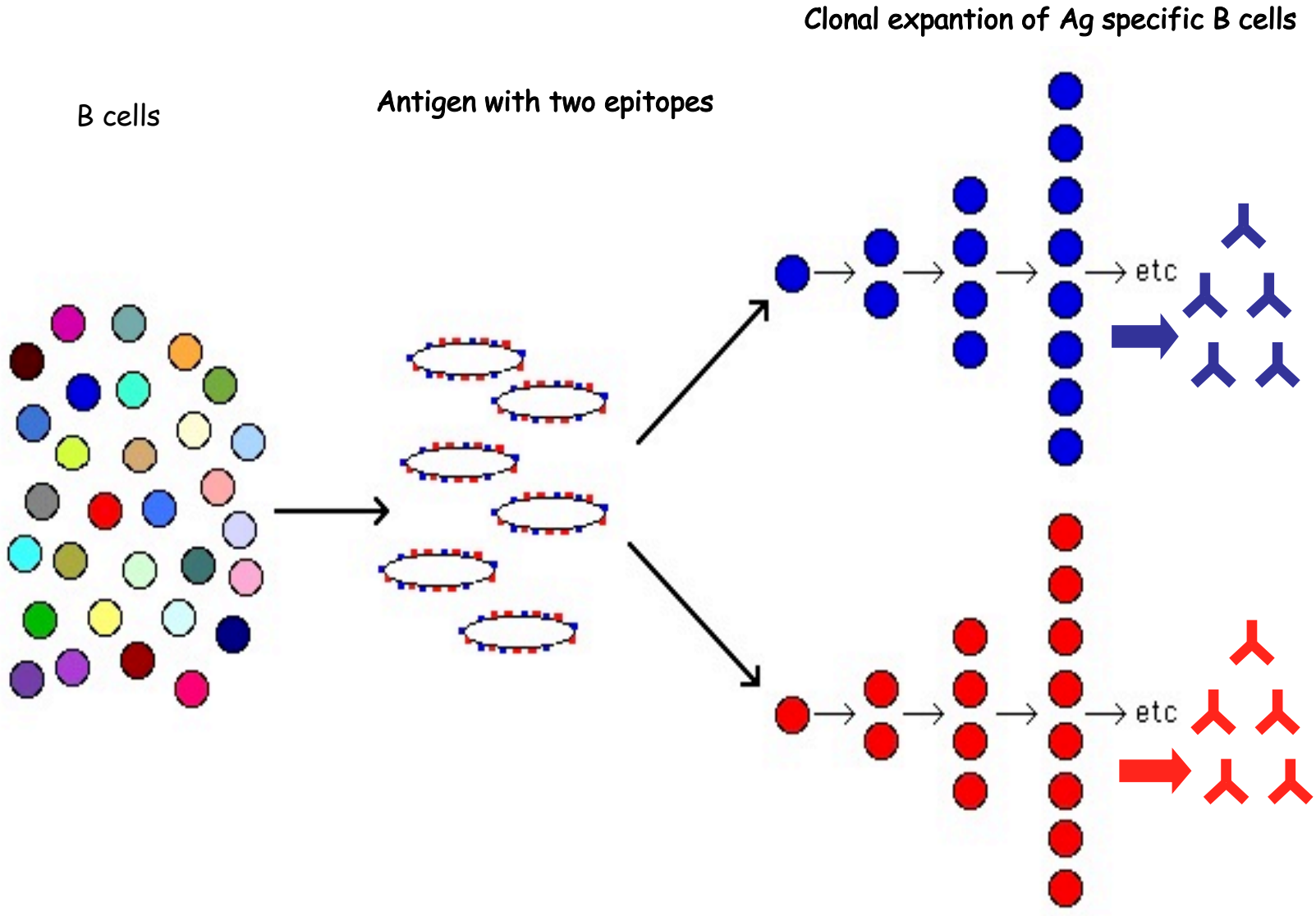
Corso di Immunologia - III anno
Prof. Paolini

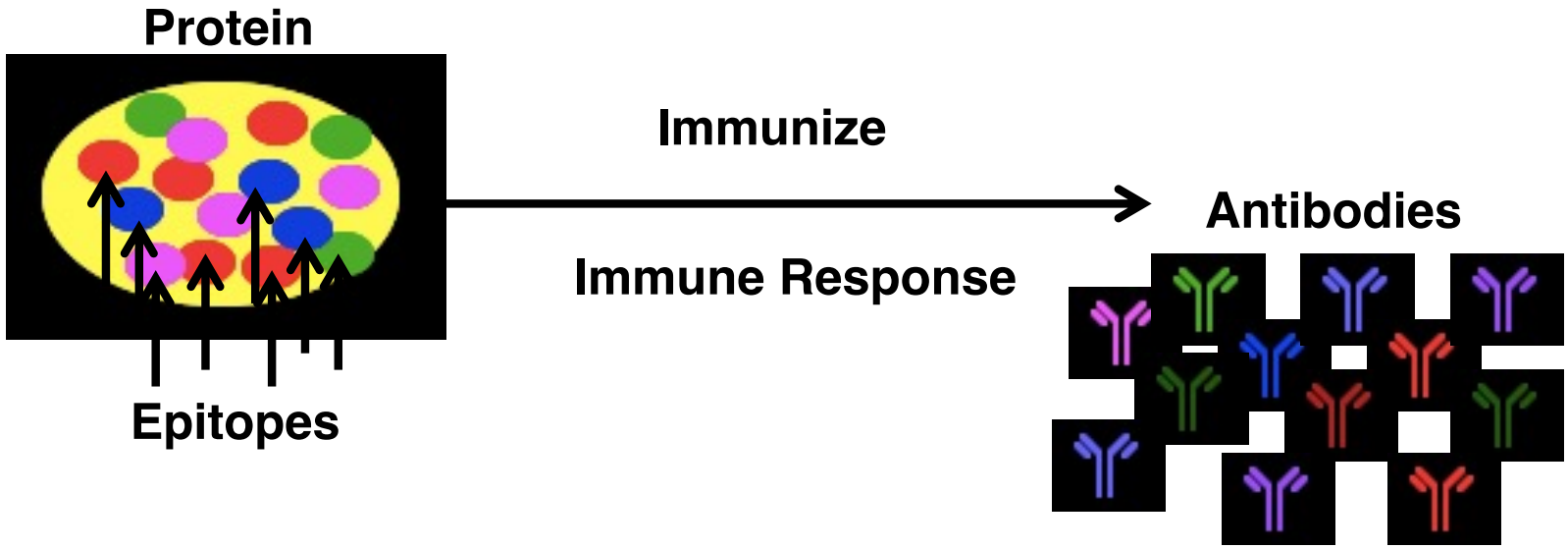
Lezione 26/11/2025

"Gli anticorpi monoclonali"

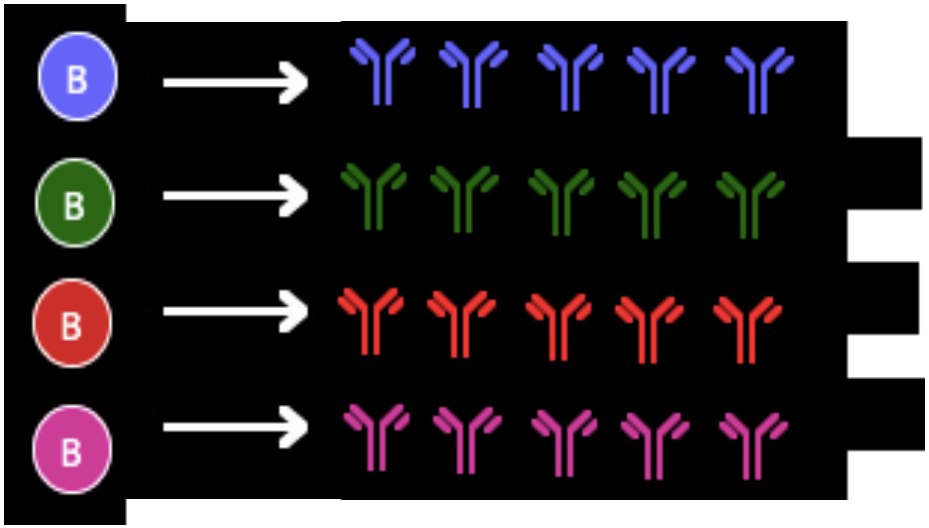


The mixture of antibodies produced in response to an antigen are referred to as polyclonal antibodies (they are produced by many different clones of B cells)

Polyclonal antibodies:



The serum obtained from an immunized animal is referred to as a polyclonal antiserum.



Polyclonal antibodies are a mixture of antibodies with different antigen binding sites that may bind to different epitopes of the immunizing agent with varying affinities.
They may be of different antibody classes.

The era of monoclonal antibodies:

the story of a discovery that revolutionized science and medicine

Köhler G, Milstein C.
Continuous cultures of fused cells secreting antibody of predefined specificity.
Nature. 1975 Aug 7

The Nobel Prize in Physiology or Medicine 1984

"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"

César Milstein

Georges J. F. Köhler

Hybridomas Technique

Antibodies produced by one single activated B cell, after immortalization by physical fusion with a myeloma cell (plasma cell tumor):

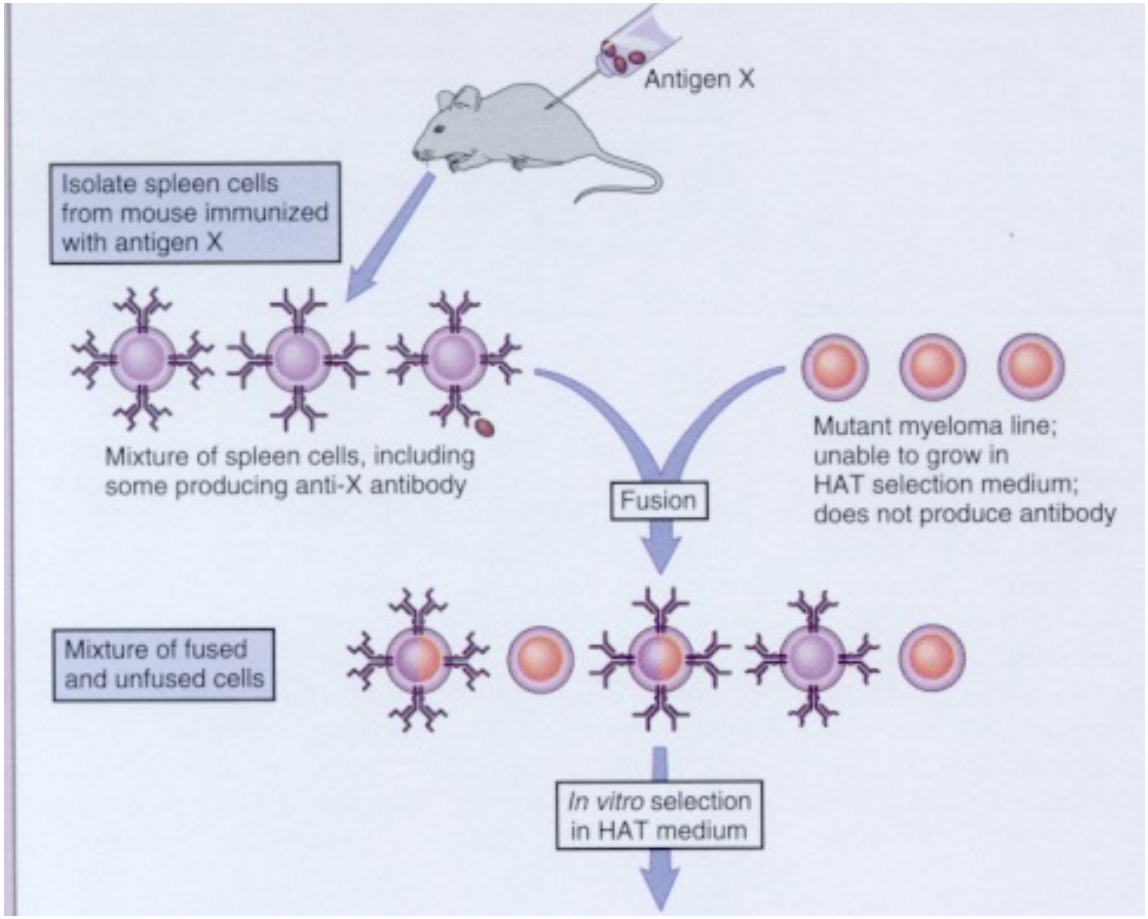
Mab are HOMOGENEOUS
- specificity
- affinity
- isotype

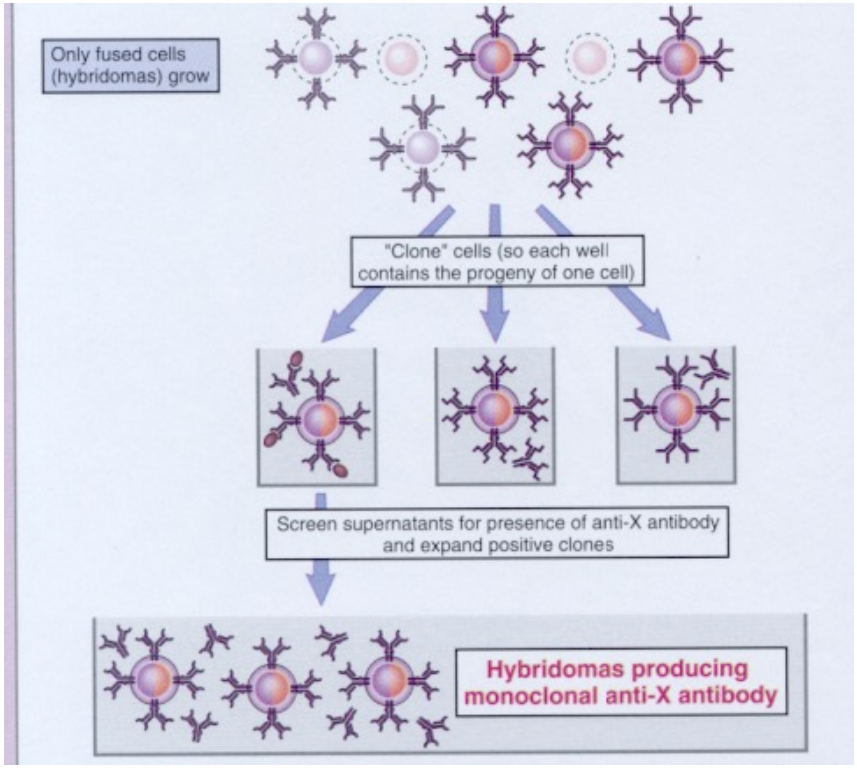
Produced in unlimited amounts, at a relatively low cost

Köhler, G., and Milstein, C.

Continuous cultures of fused cells secreting antibodies of predefined specificity.

Nature, 256: 995, (1975).





Characteristics of the myeloma cell partner

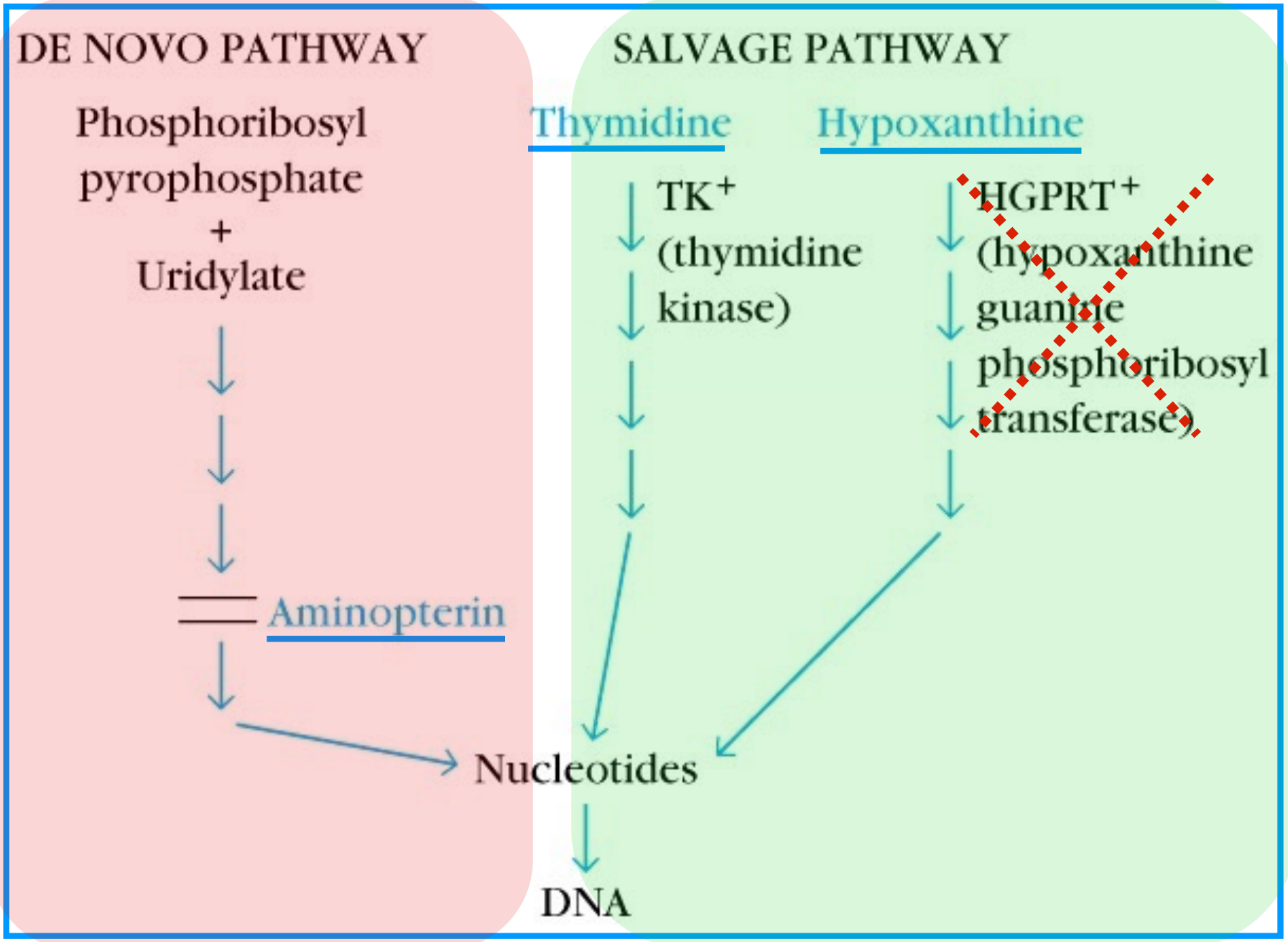
1. Immortal

2. Does not produce Ig (mutant)

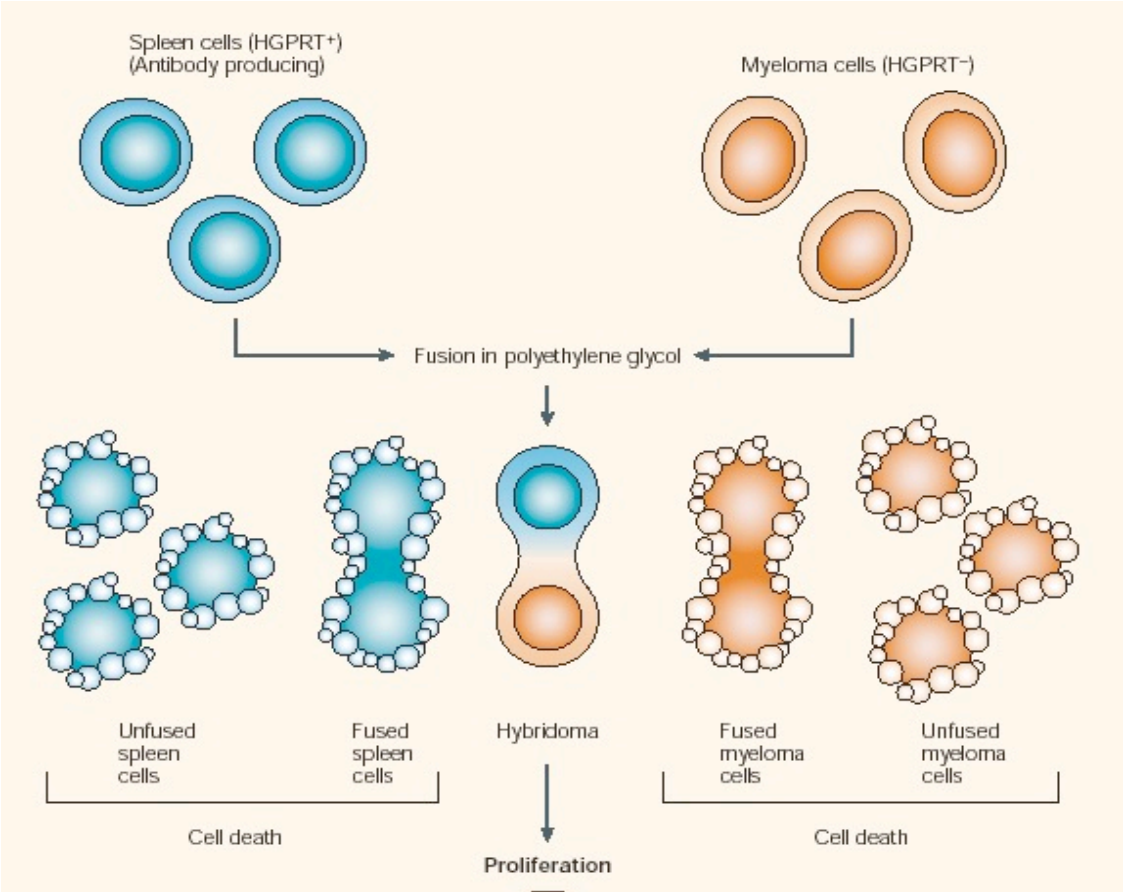
3. Lacks HGPRT (hypoxanthine guanine phosphoribosyltransferase), thus:

4. Cannot grow in HAT selective medium (Hypoxanthine, Aminopterin, Thymidine)

Pathways for nucleotide biosynthesis



Hybridoma Selection: The "HAT Trick"



Myeloma cells lack certain enzymes so that they cannot use hypoxanthine and thymidine as a source for nucleic acid biosynthesis and will die in culture when grown in HAT medium.

Only myeloma cells that have fused with B cells will survive

The hybridoma technique for monoclonal antibody production (1)

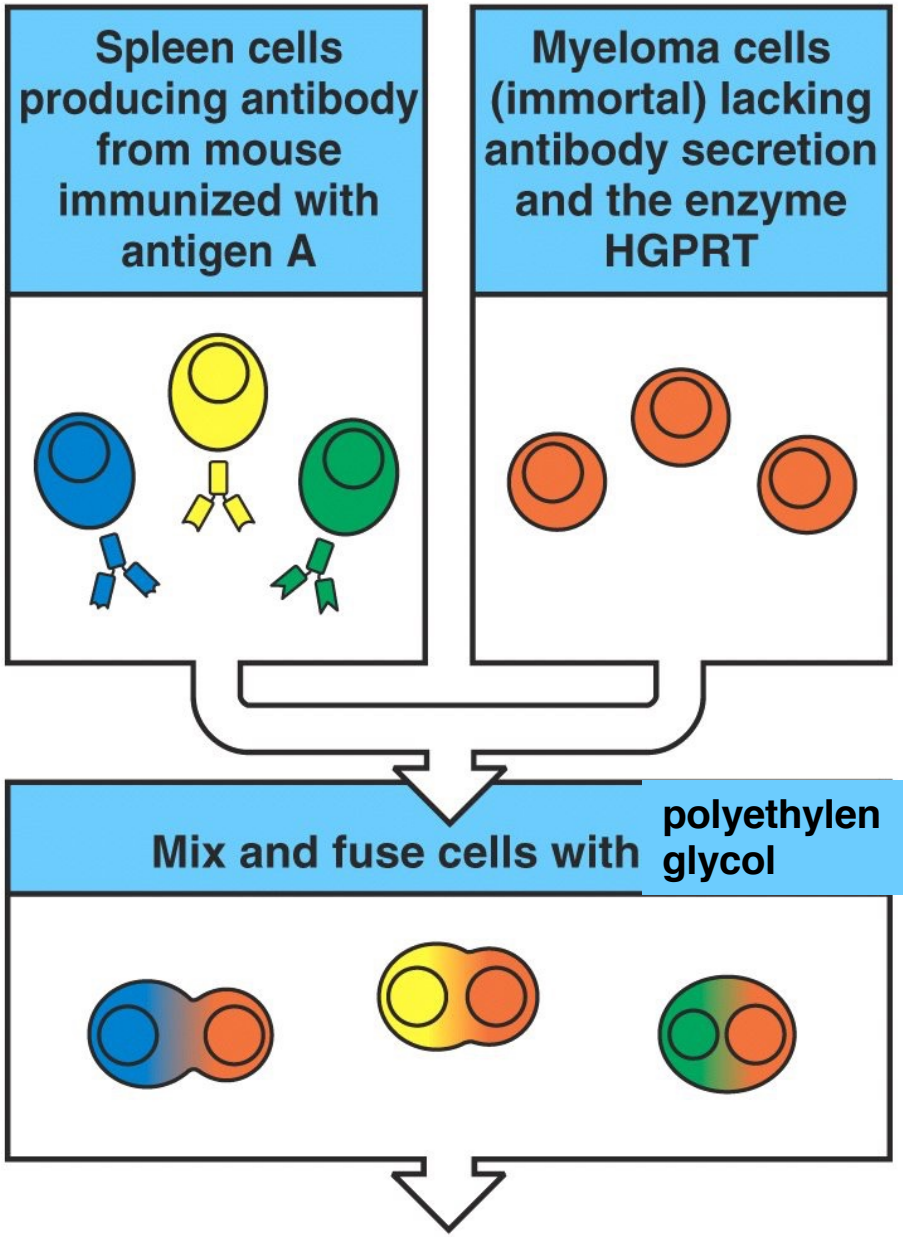


Figure A-14 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

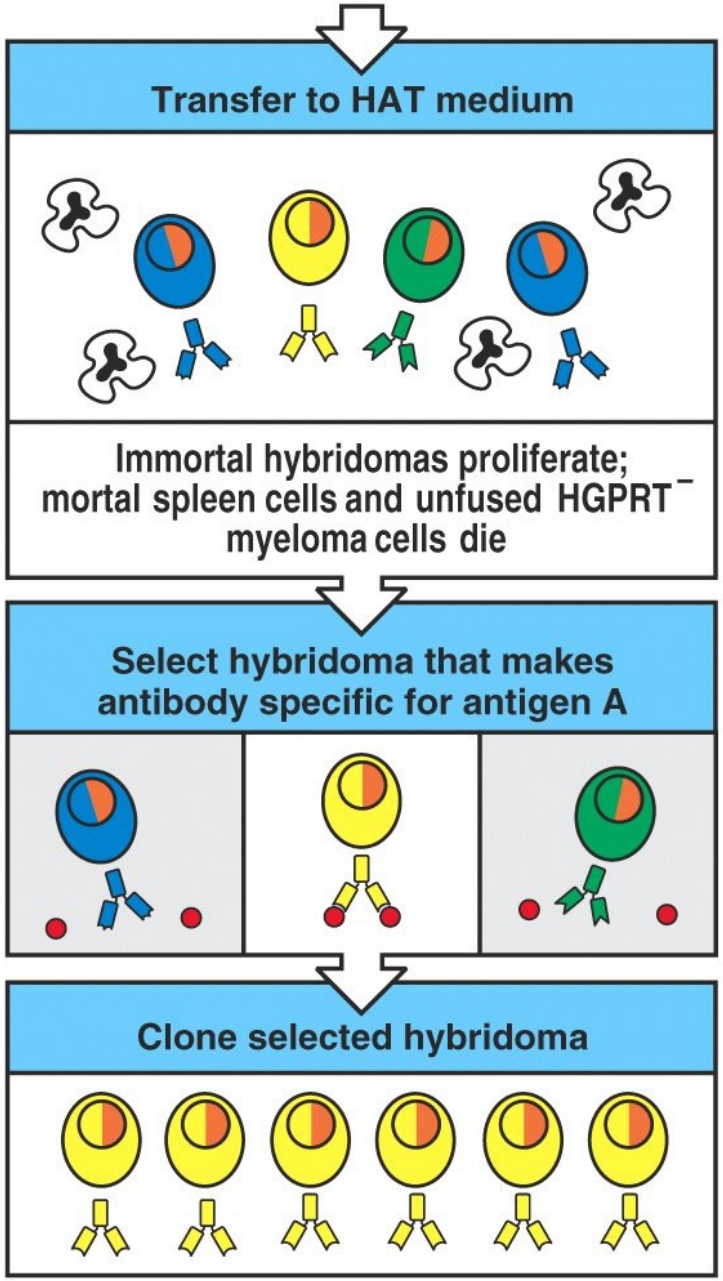
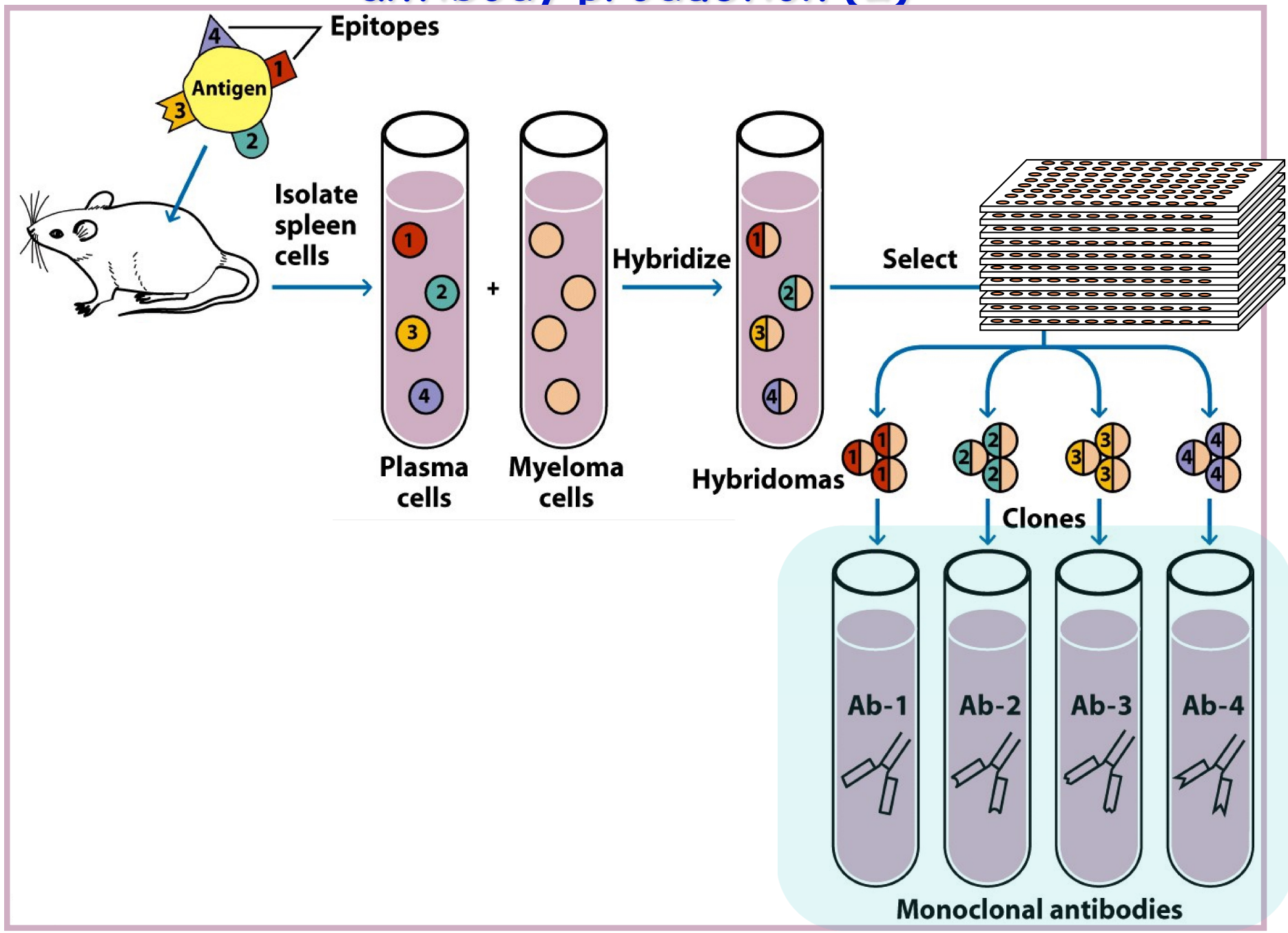
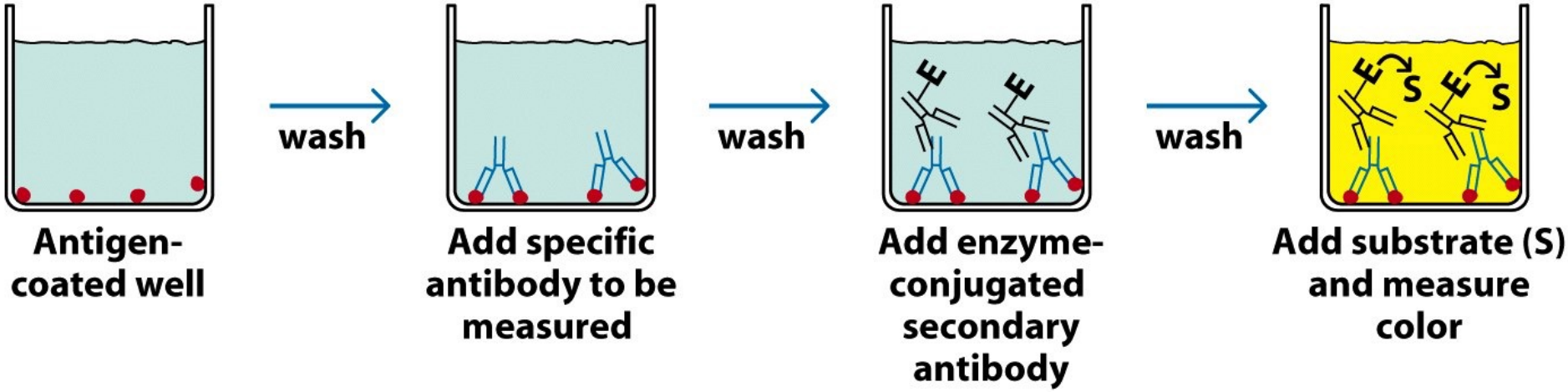


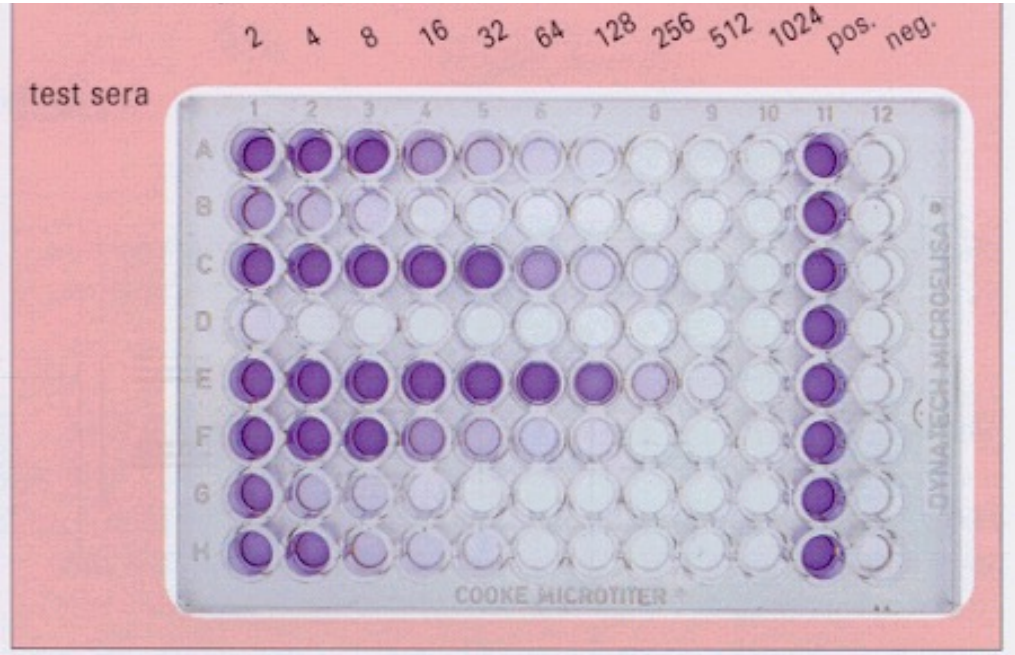
Figure A-14 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

The hybridoma technique for monoclonal antibody production (2)

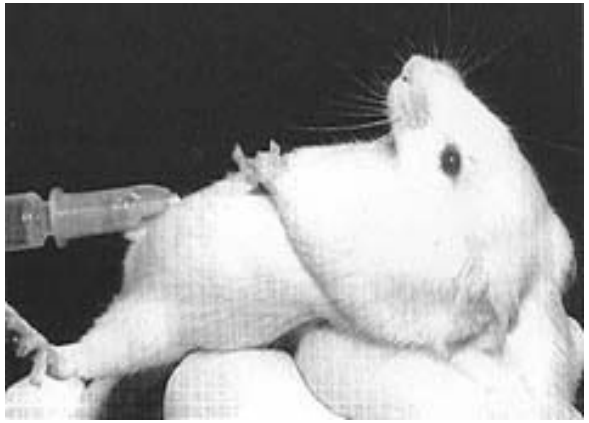


Enzyme-linked immunosorbent assay (ELISA)





PRODUCTION OF MONOCLONAL ANTIBODIES



Yesterday…..

Generation of ascites containing high amounts of mAbs (5-20 mg/ml)

……Today

Bioreactors (5-10 mg/ml)

Applications of monoclonal antibodies

In vitro

Identification and quantitation of an enormous range of molecules in almost any biological sample

Identification of cell subsets (lineage, differentiation step, activation state, functional capability) through the analysis of specific markers

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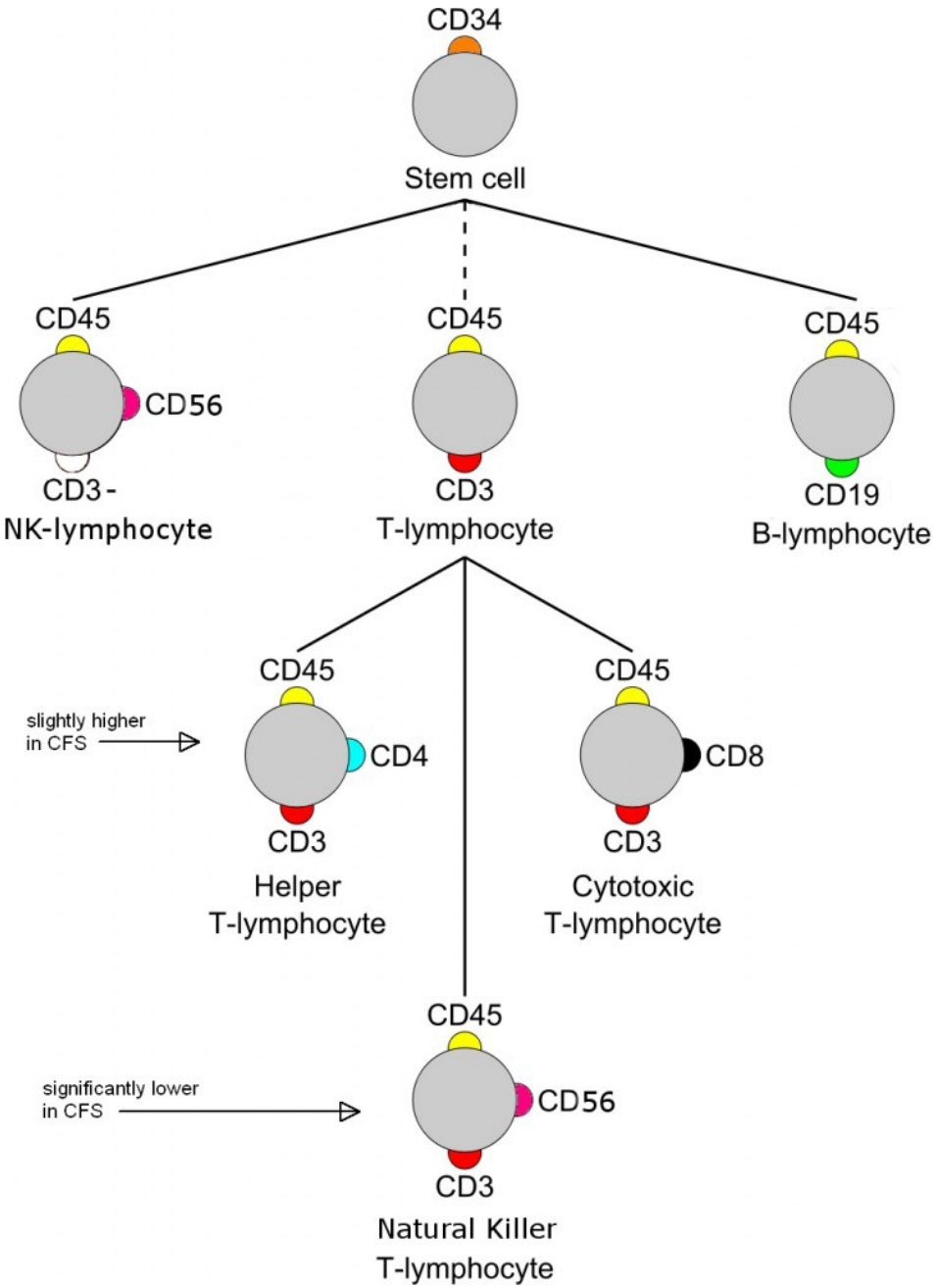
In vivo

Diagnosis, imaging

Immunotherapy:
tumors, transplantation, autoimmune diseases, infectious diseases

Anti-CD45	Leucocytes
Anti-CD3 Anti-CD4 Anti-CD8	T cells
Anti-CD19	B cells
Anti-CD56 Anti-CD16	NK cells

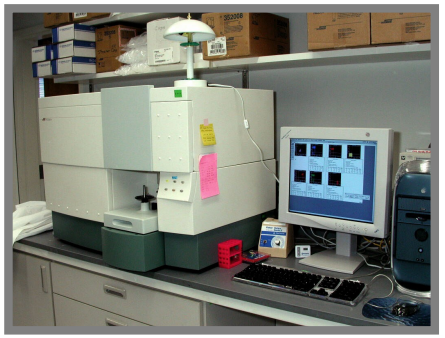


The principle of flow cytometry

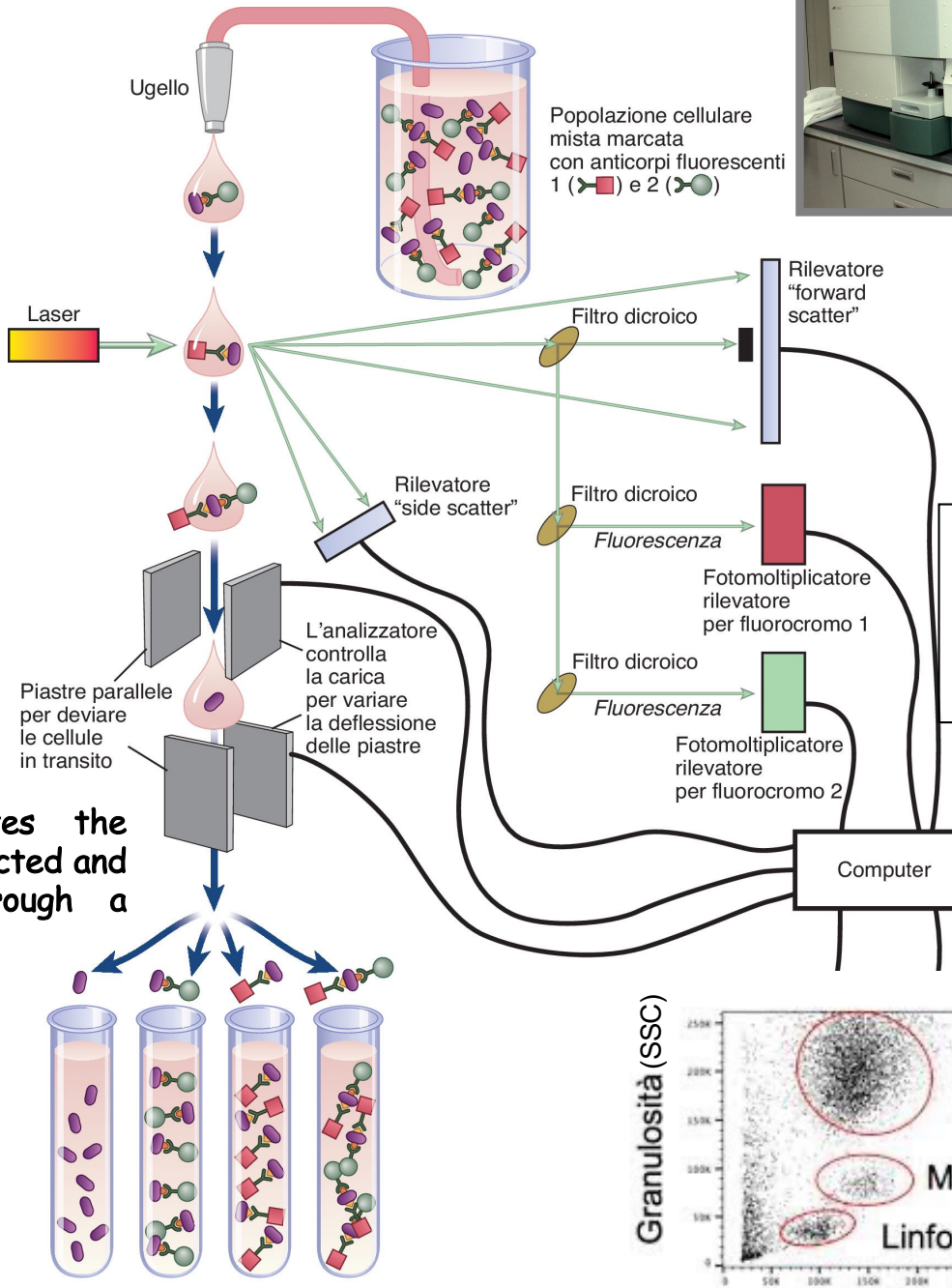
Fluid system for sample transport

Excitation system

Detection system



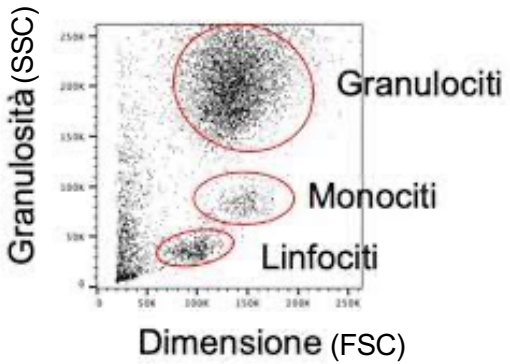
Each single cell is hit by a beam of light which excites the fluorochrome and determines the emission of a fluorescent signal

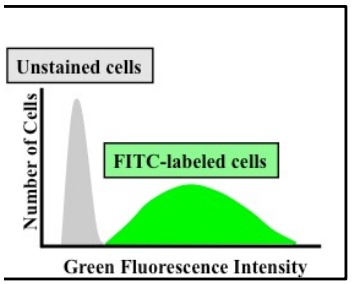


The light signal passes through a system of filters and mirrors and is amplified and converted into an electrical signal that is finally sent to a data analyzer.

Electrical system

Through deflection plates the analyzed cells can be deflected and collected separately through a process called "sorting"

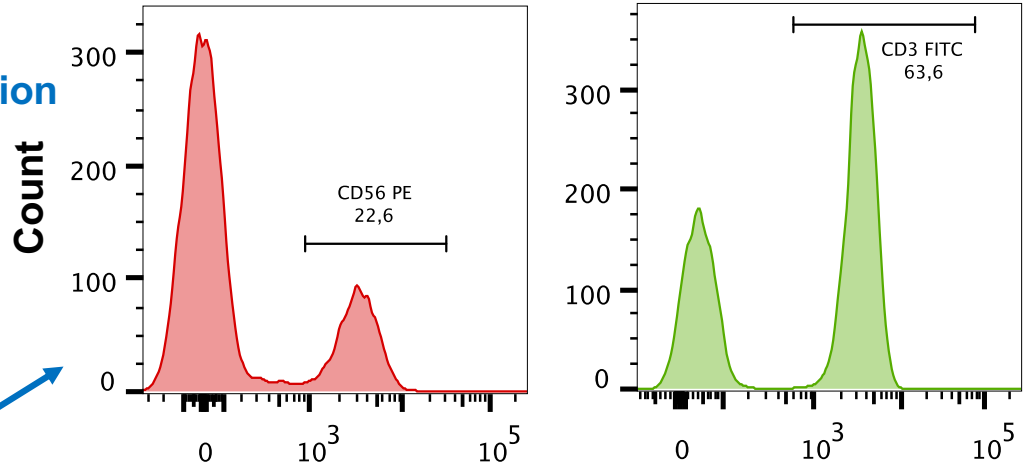


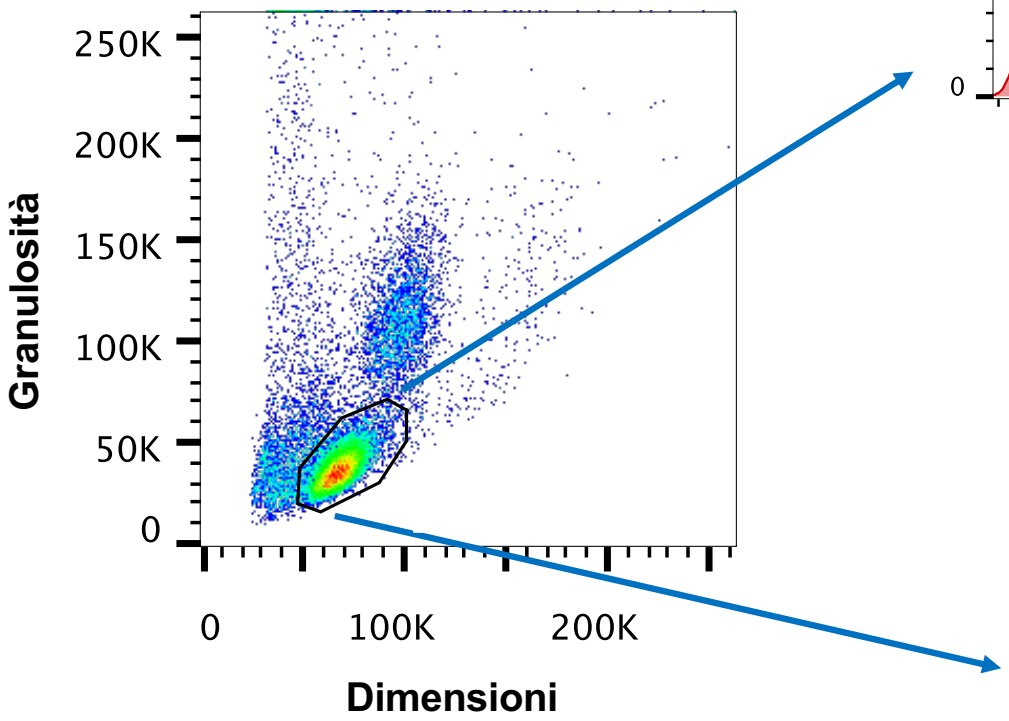


GRAPHIC REPRESENTATION OF FLUORESCENCE SIGNALS

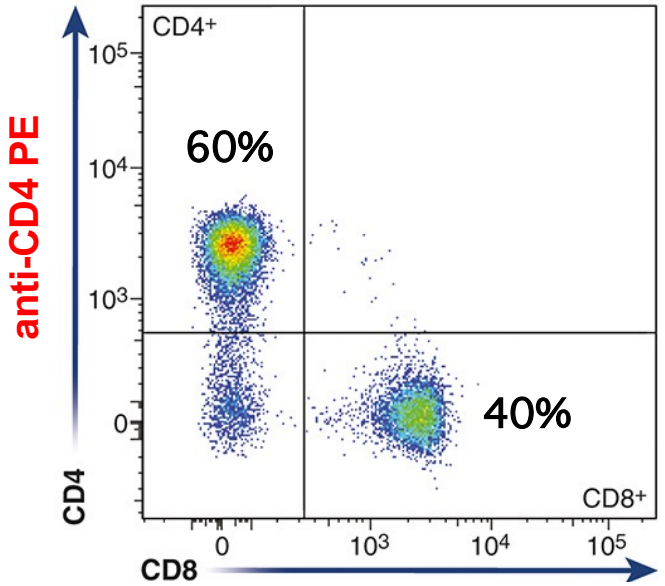
1. SINGLE PARAMETER ANALYSIS

The histogram is the graphical representation of a single distribution (a fluorescence signal)





2. MULTIPARAMETRIC ANALYSIS



Il citogramma di dispersione a puntini (dot plot) è la rappresentazione grafica di due distribuzioni (due segnali di fluorescenza)

Monitoring of leukocytes during pathologies or therapies:

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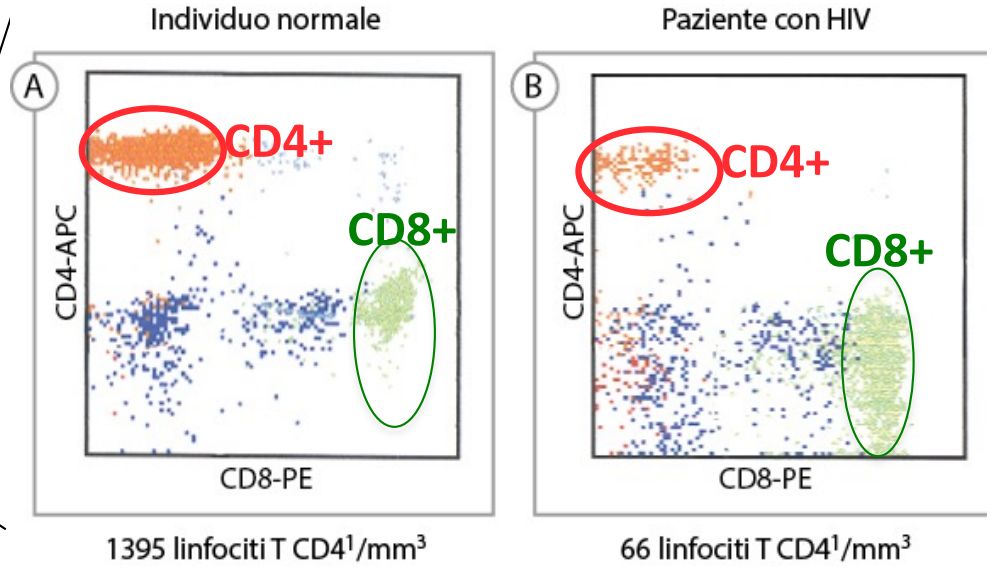
Examples of pathological situations associated with an altered composition of lymphocyte subpopulations in the blood

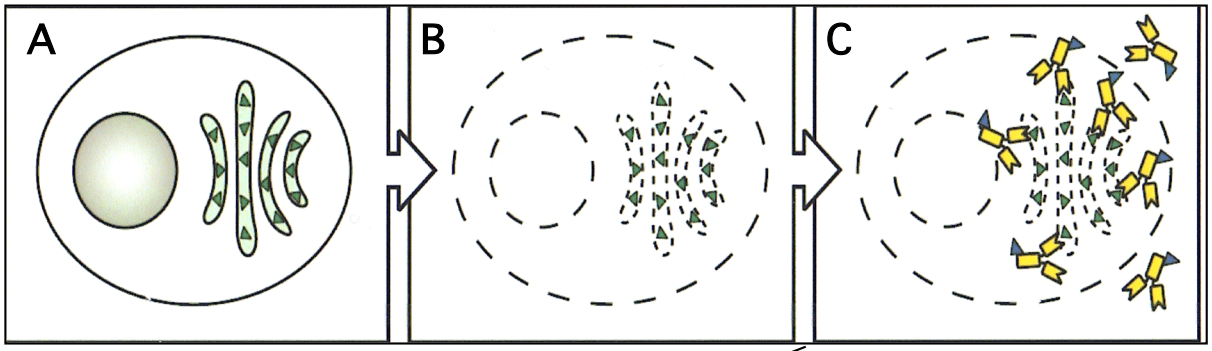
Condition	Main finding[a]	
Diseases		
Skin:		
candidiasis	$CD4^+$	↓
Kala Azar	$CD4^+$	↓
Nervous system:		
Down syndrome	$CD4^+CD45R^+$	↑
multiple sclerosis	$CD4^+CD45R^+$	↓
schizophrenia	$B\ CD5^+$	↑
Respiratory system:		
pneumonia	$CD4^+$	↓
pulmonary sarcoidosis	$CD8^+$	↑
Alimentary system:		
coeliac disease	$CD4^+$	↓
Crohn's disease	$CD8^+$	↓
Urogenital system:		
pyelonephritis	$CD4^+$	↓
Immune system:		
AIDS	$CD4^+$	↓
Rush hyposensitization	$CD4^+CD45R^+$	↑
Endocrine system:		
Graves' disease	$CD8^+DR^+$	↑
Hashimoto's disease	$CD4^+DR^+$	↑
Hematopoietic system:		
idiopathic thrombocytopenic purpura	$CD4^+$	↓
Joints and connective tissues:		
systemic lupus erythematosus	$CD4^+CD45^+$	↓

[a] ↑ increase, ↓ decrease in the numbers in the subset

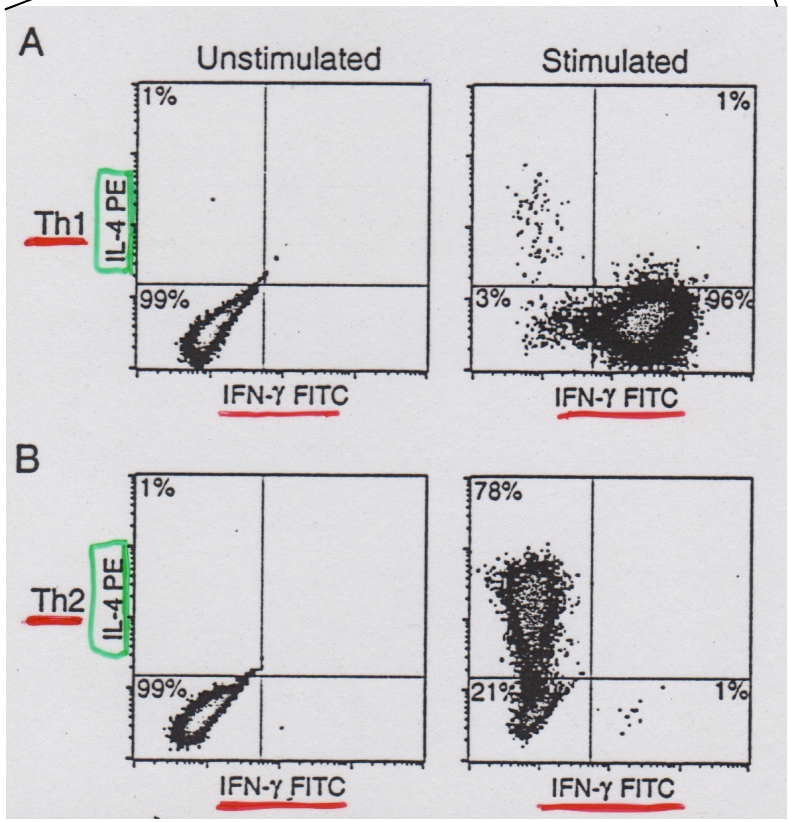
Flow Cytometry Analysis of CD4+ and CD8+ T Lymphocytes from a Patient with HIV Infection



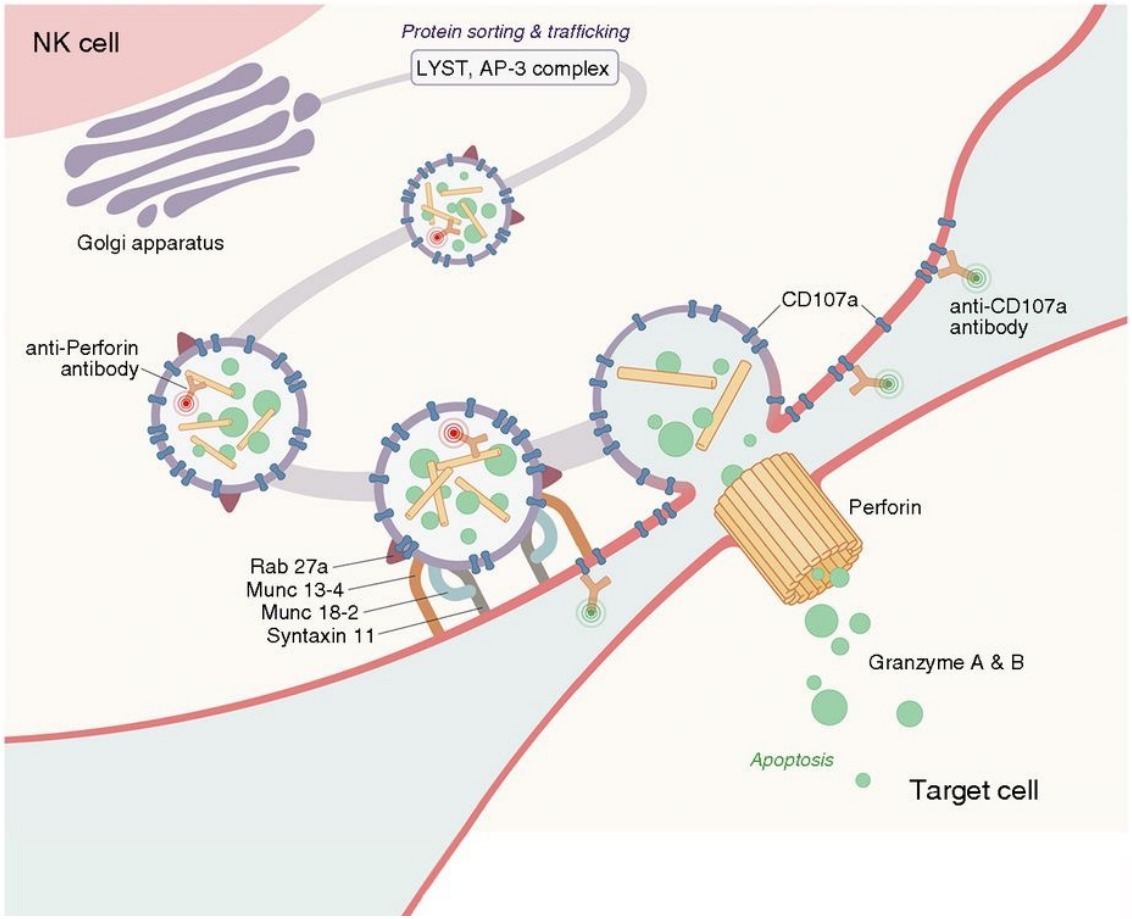
Monitoring the functional status of immune system cells: cytokine production

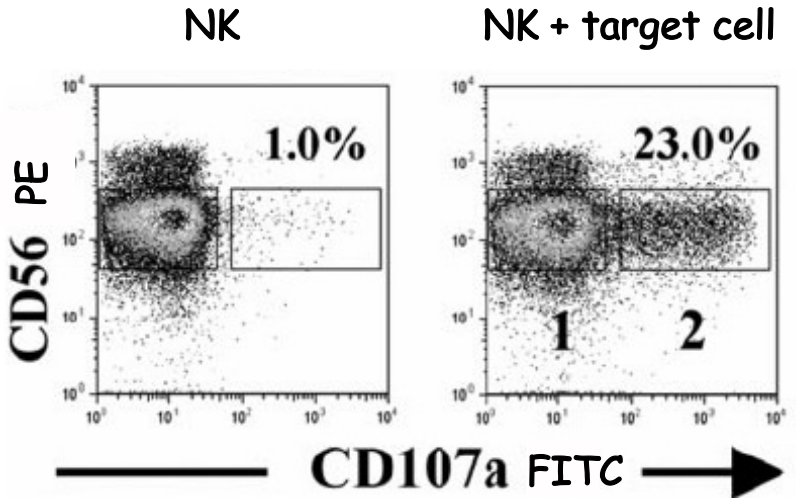


1. Stimulation
2. Blocking exocytosis
3. Fixation
4. Permeabilization
5. Intracellular staining

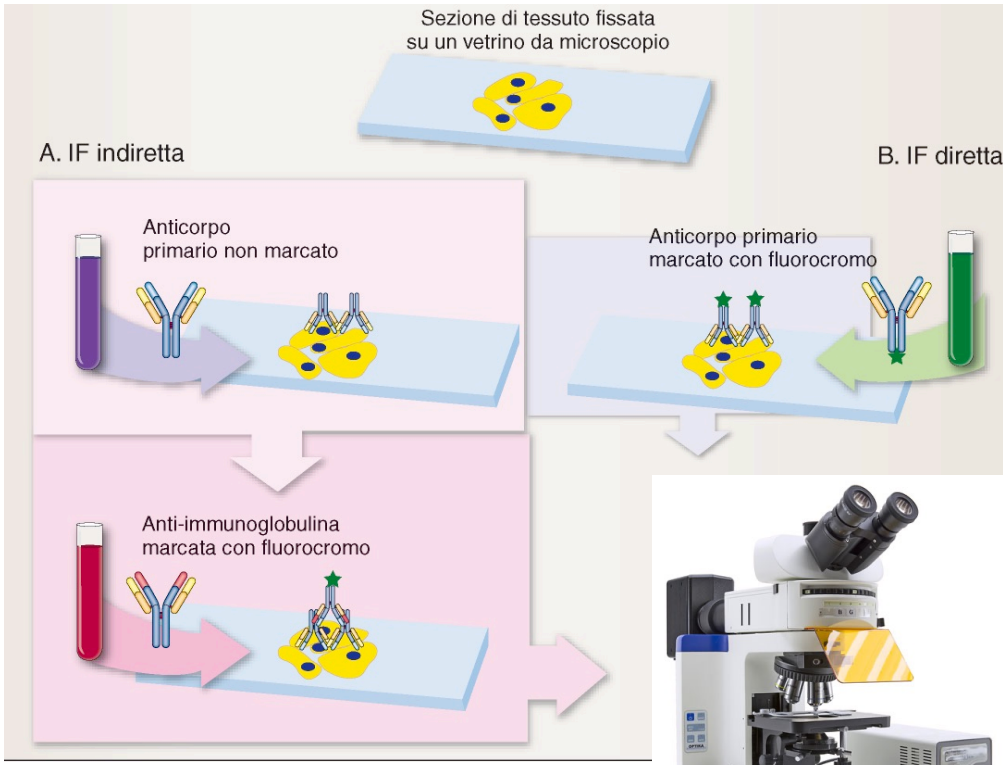


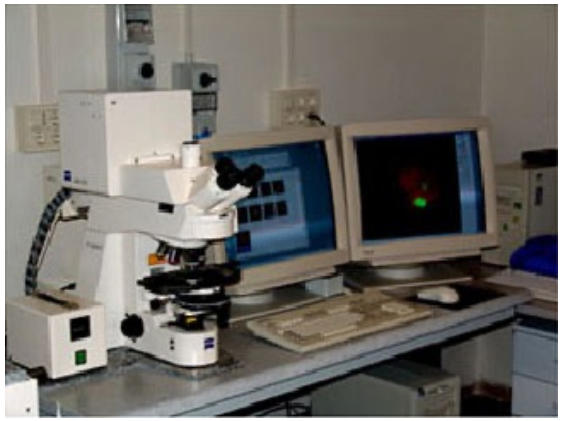
Monitoring the functional status of immune cells: cytotoxicity tests





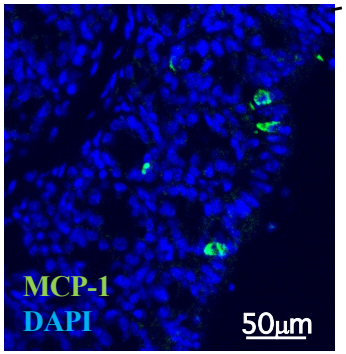
Tissue distribution assessment: fluorescence and confocal microscopy

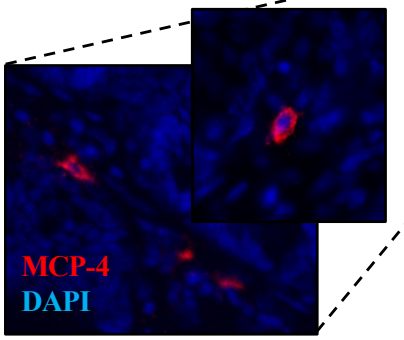




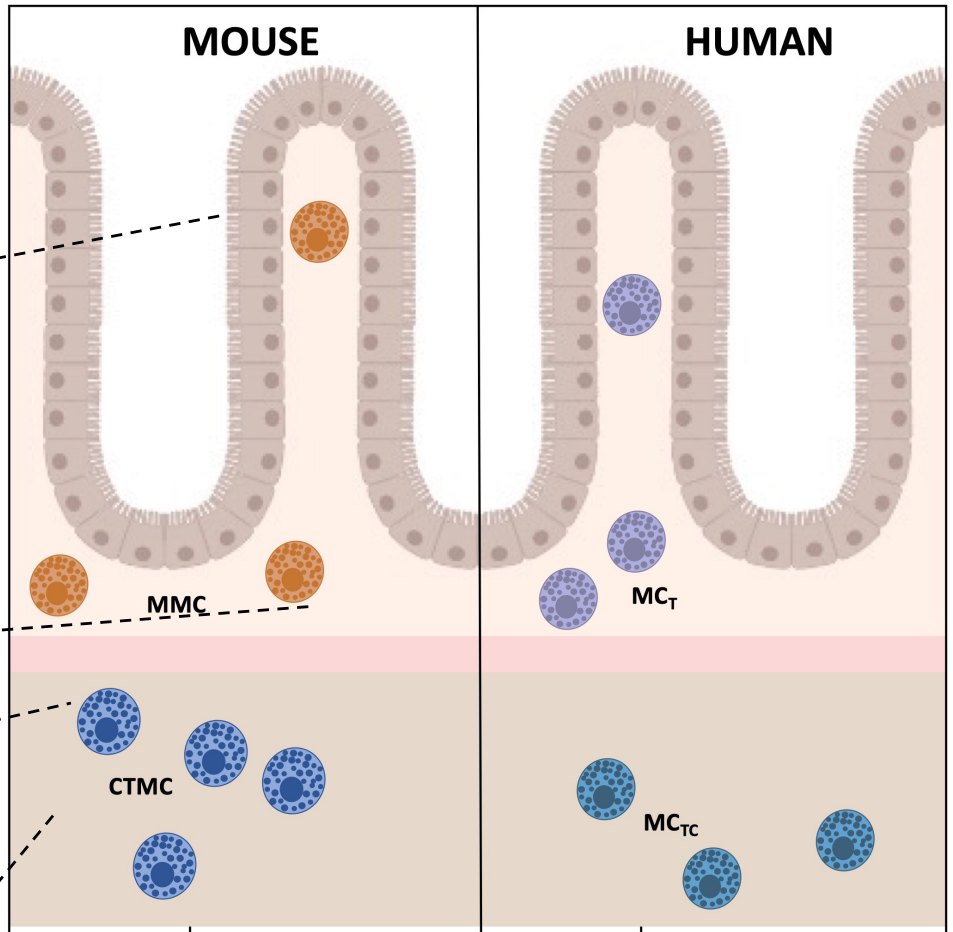
Intestinal mast cells include two subsets that express different proteases in their granules

MCP-1 = tryptase expressed in the granules of mucosal mast cells

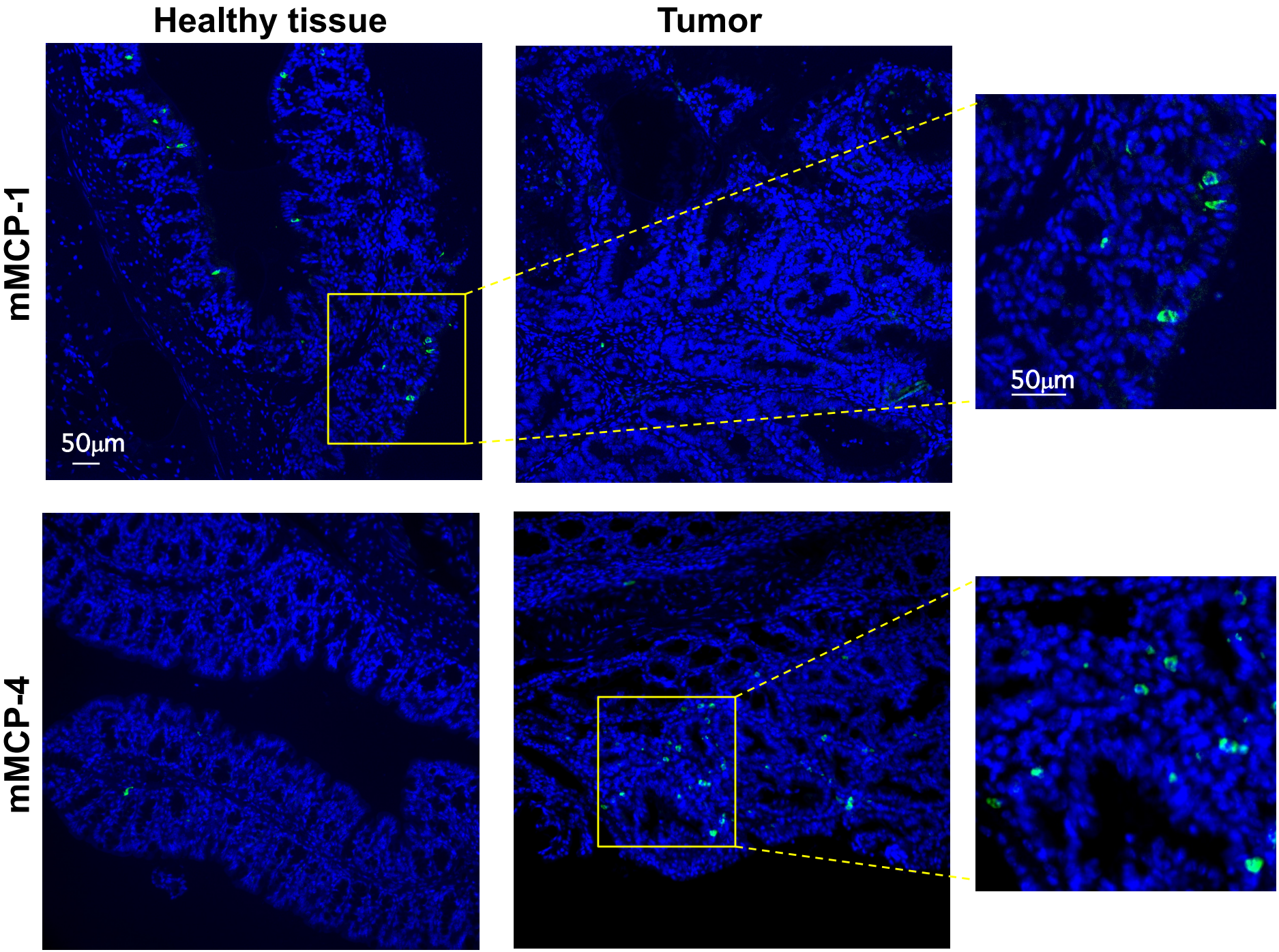




MCP-4 = chymase expressed in mast cell granules of the connective tissue



	Proteases		Proteases
MMC	mMCP-1,2	MC$_T$	Tryptase
CTMC	mMCP-4,5,6,7	MC$_{TC}$	Tryptase, Chymase


Healthy tissue
Tumor
mMCP-1
mMCP-4
50μm
50μm

Applications of monoclonal antibodies

In vitro

Identification and quantitation of an enormous range of molecules in almost any biological sample

Identification of cell subsets (lineage, differentiation step, activation state, functional capability) through the analysis of specific markers

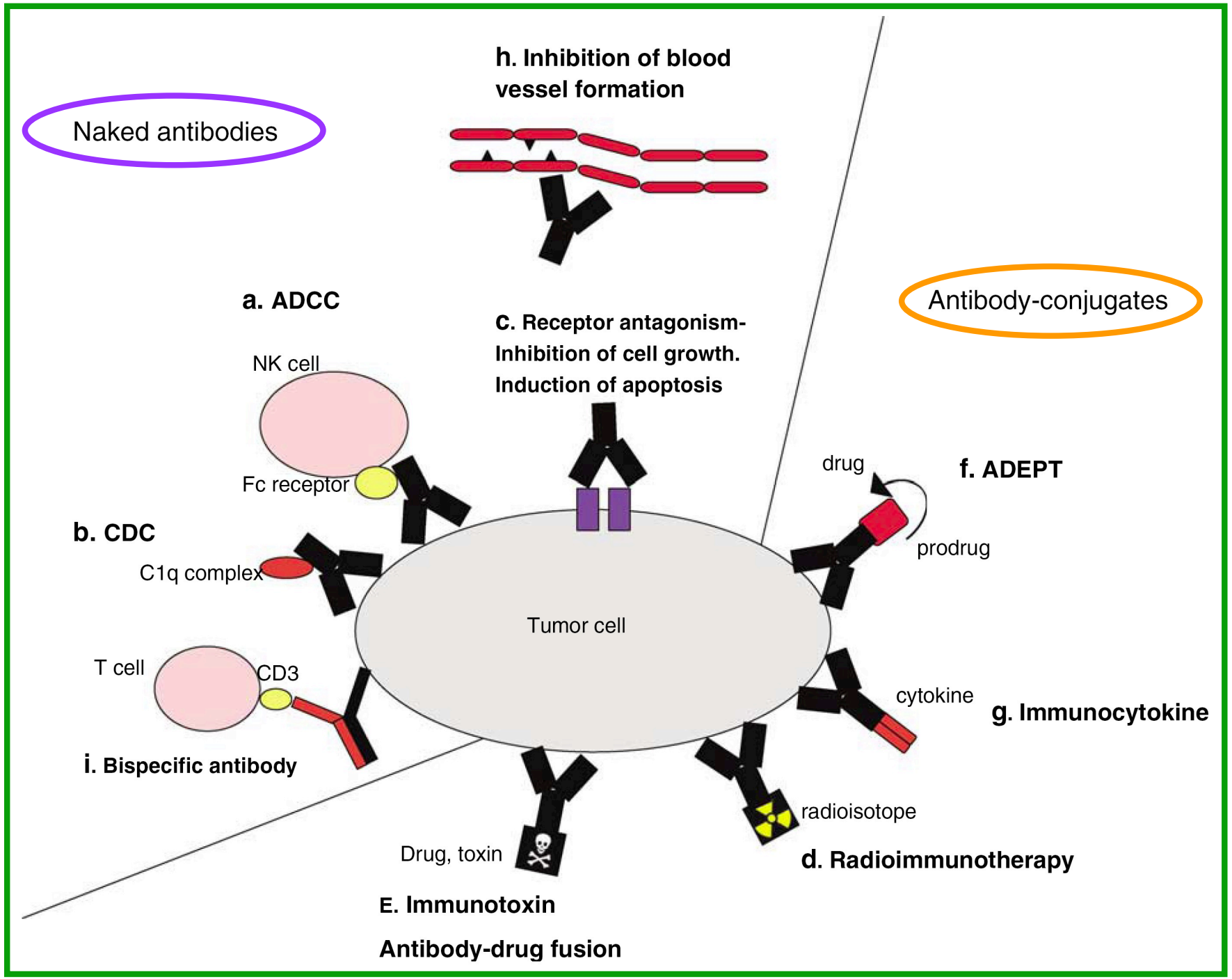
IMMUNODIAGNOSIS

In vivo

Diagnosis, imaging

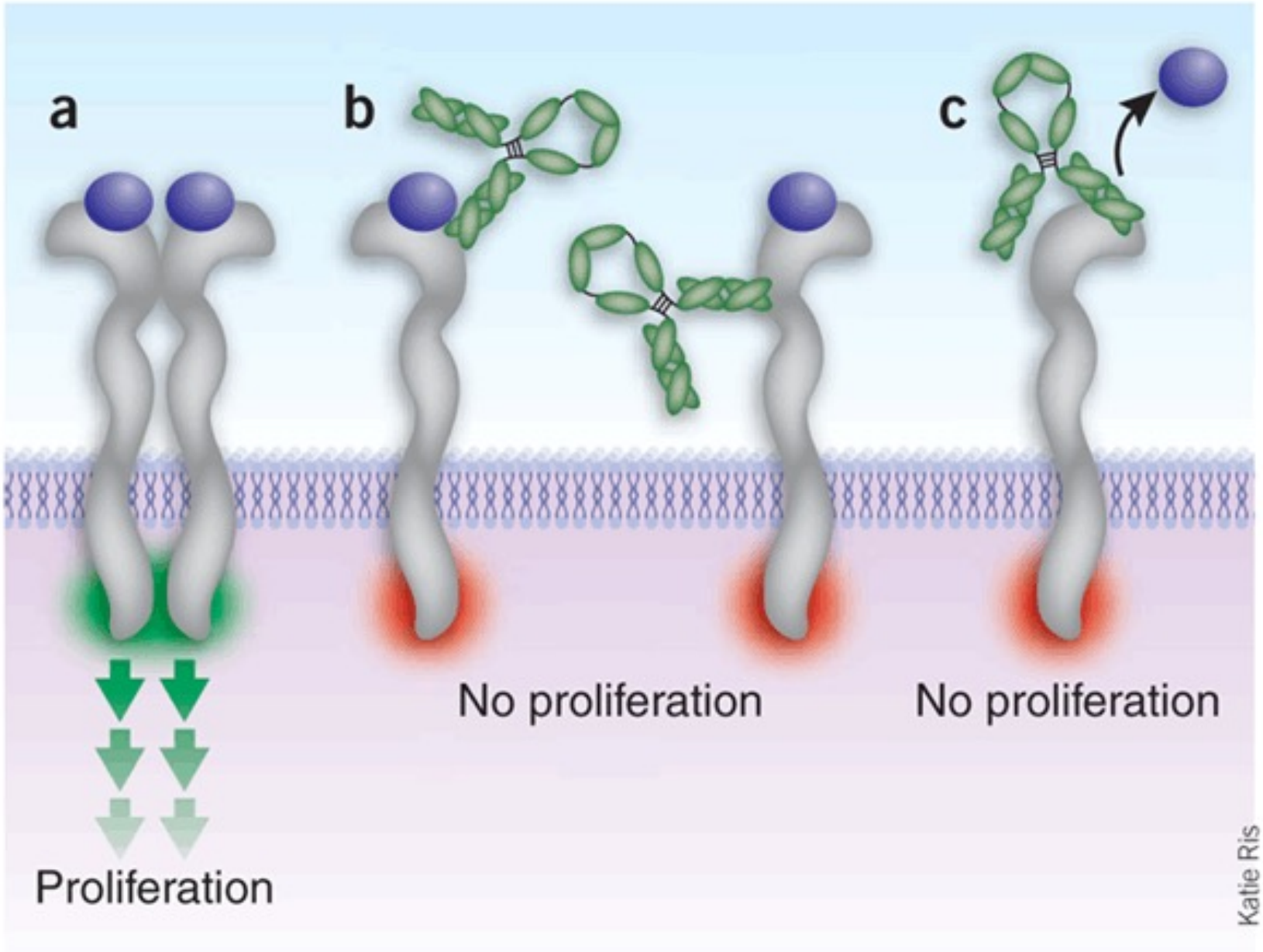
Immunotherapy:
tumors, transplantation, autoimmune diseases, infectious diseases

Antitumor mechanisms of action of therapeutic antibodies



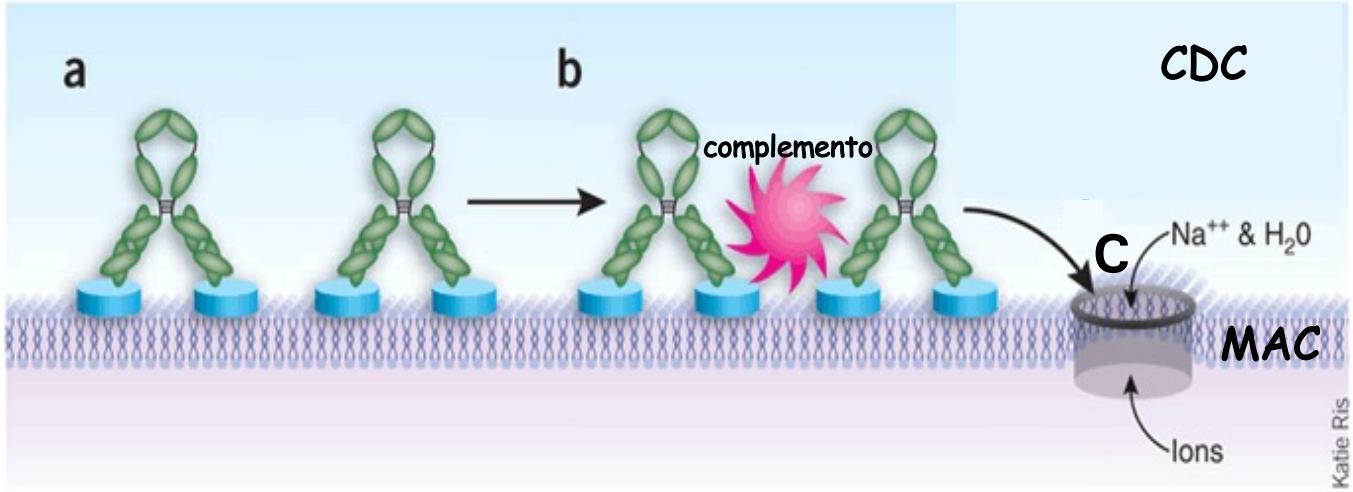
Action by naked antibody:

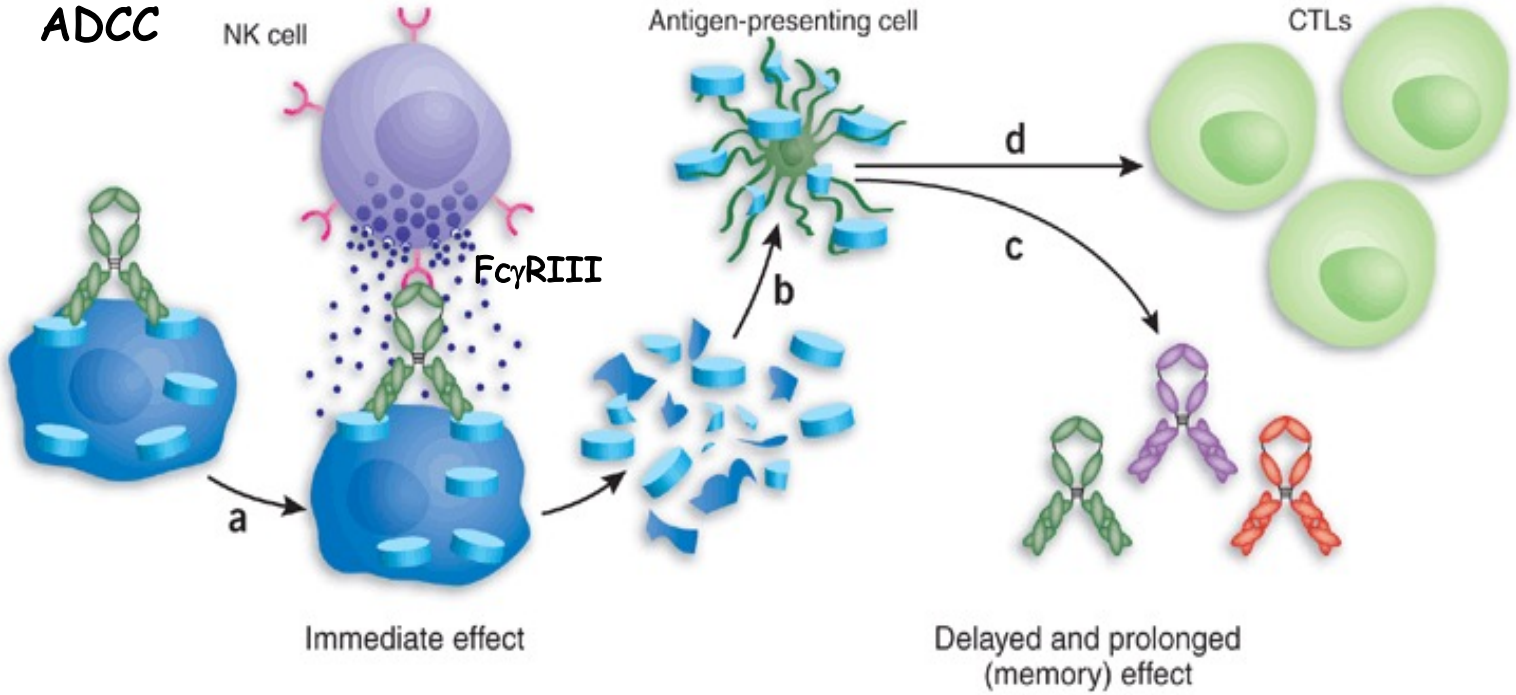
Antibody-mediated signaling inhibition



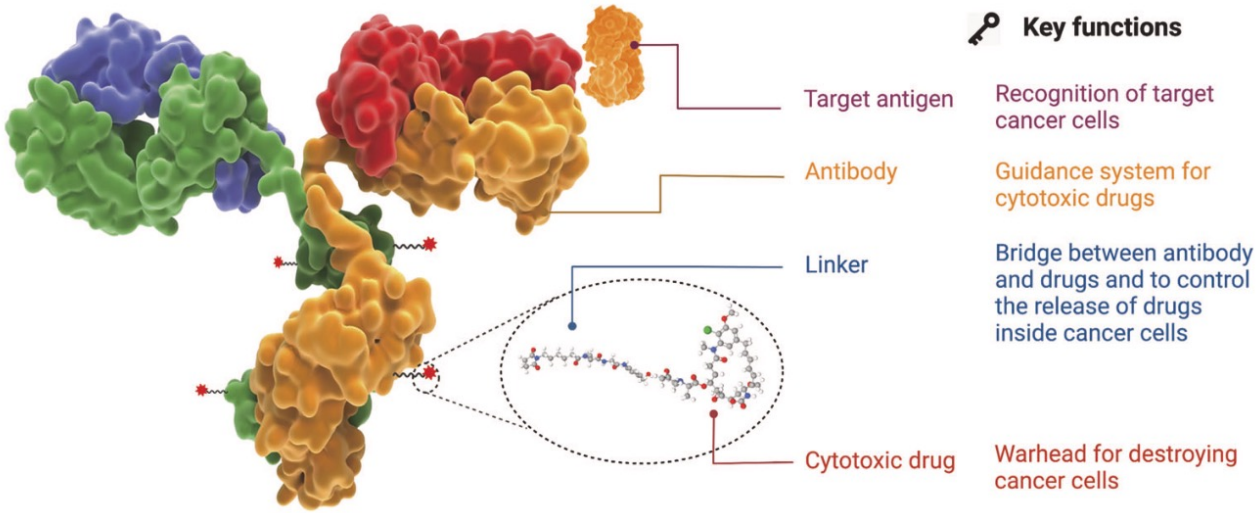
Action by naked antibody:

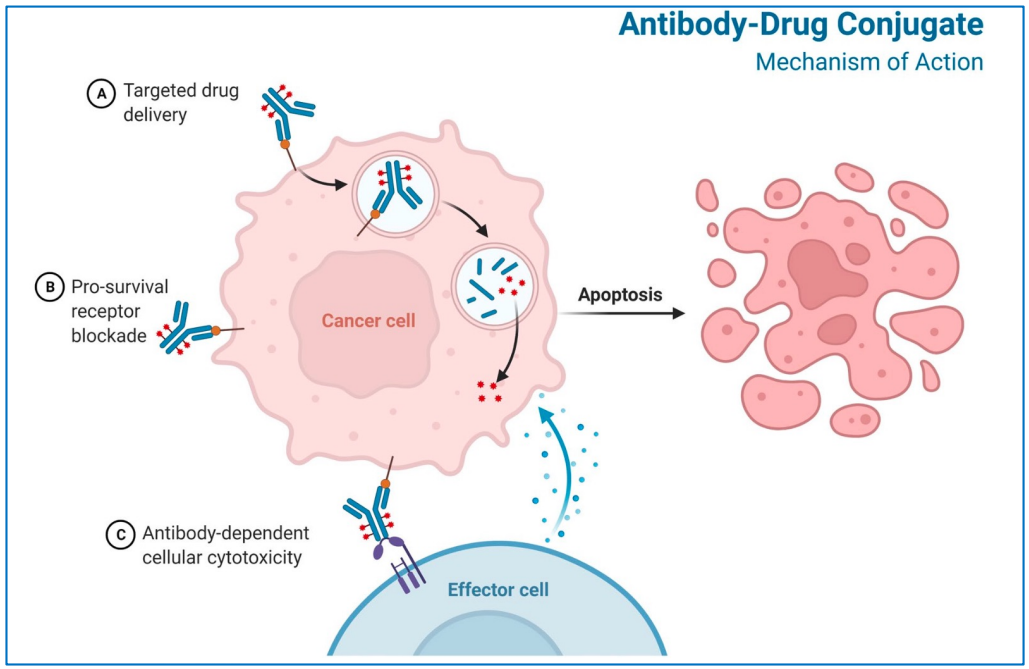
Complement-directed cytotoxicity (CDC) and ADCC-mediated cytotoxicity





Antibody drug conjugate (ADC)

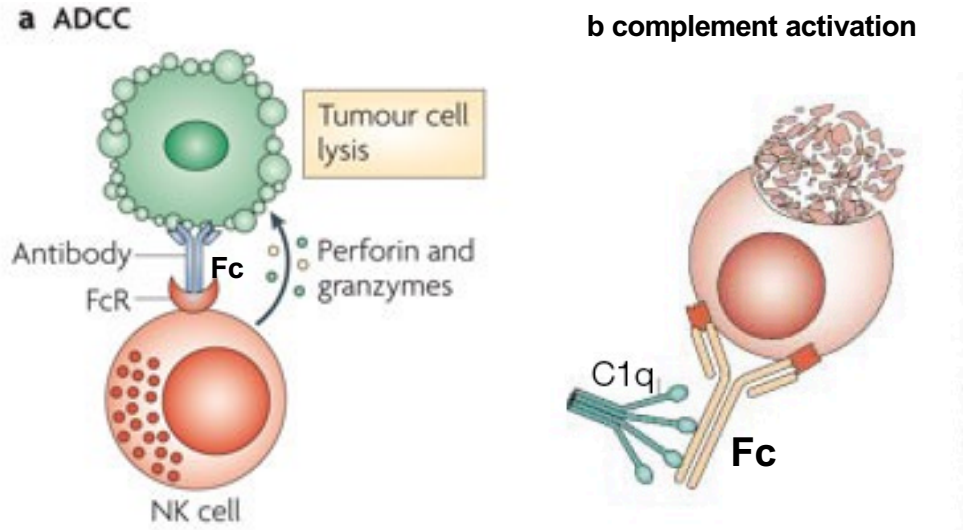




Obstacles to the *in vivo* use of therapeutic mAb

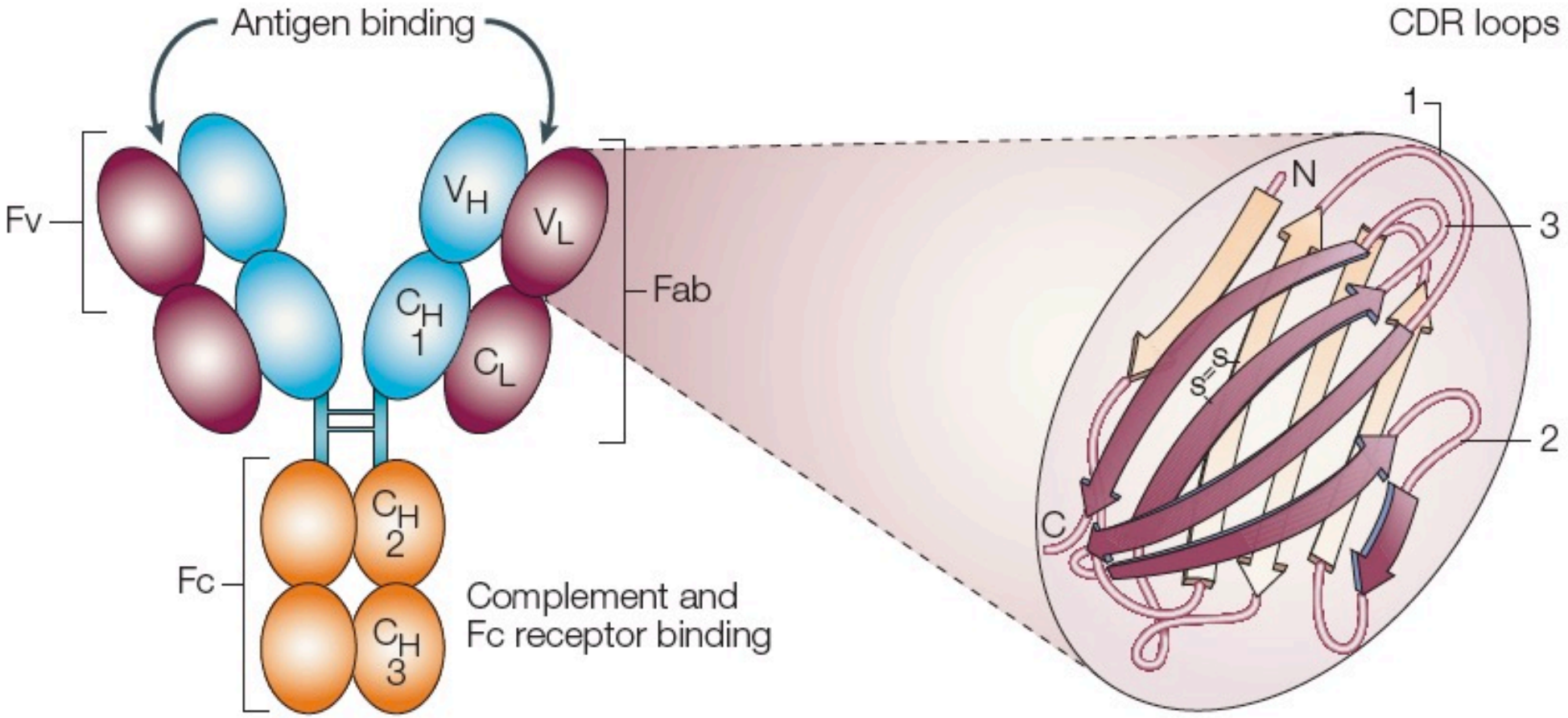
•HAMA (Human Anti-Mouse Antibodies) responses

•Murine mAb do not effectively activate Fc-mediated effector functions (ADCC, attivazione del complemento)

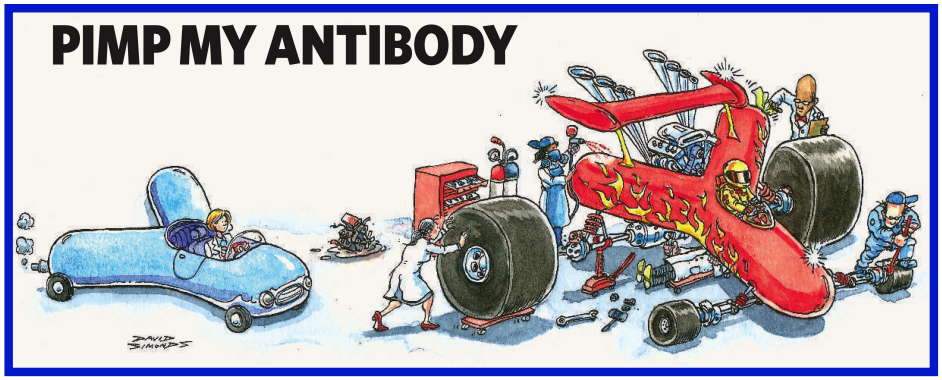


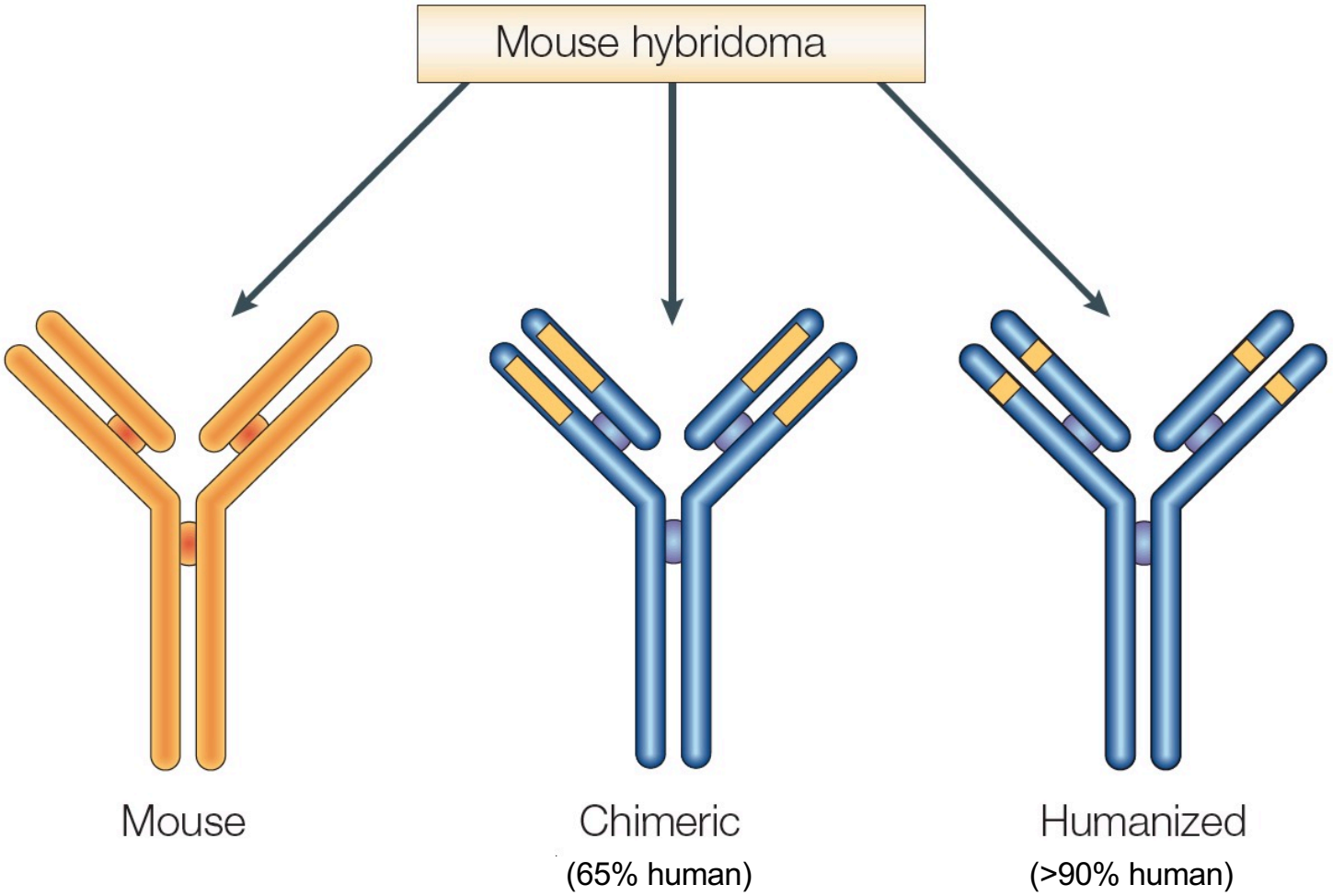
•Murine Abs have a shorter half-life than human Ab have (FcRn-Brambell receptor -binding on endothelial cells, continuous Ig recycling)

The modular structure of immunoglobulins

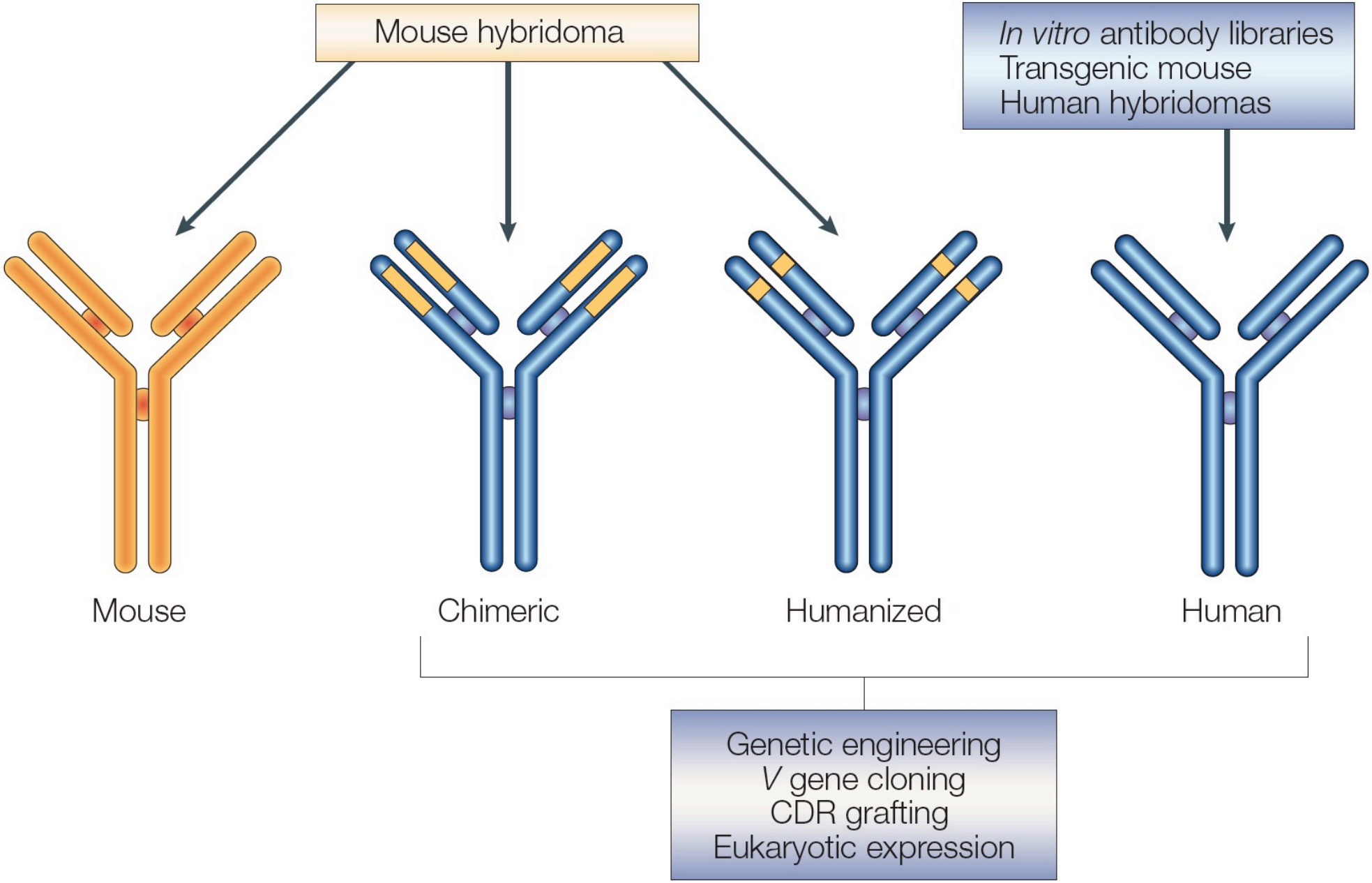


Monoclonal antibodies can be genetically manipulated!

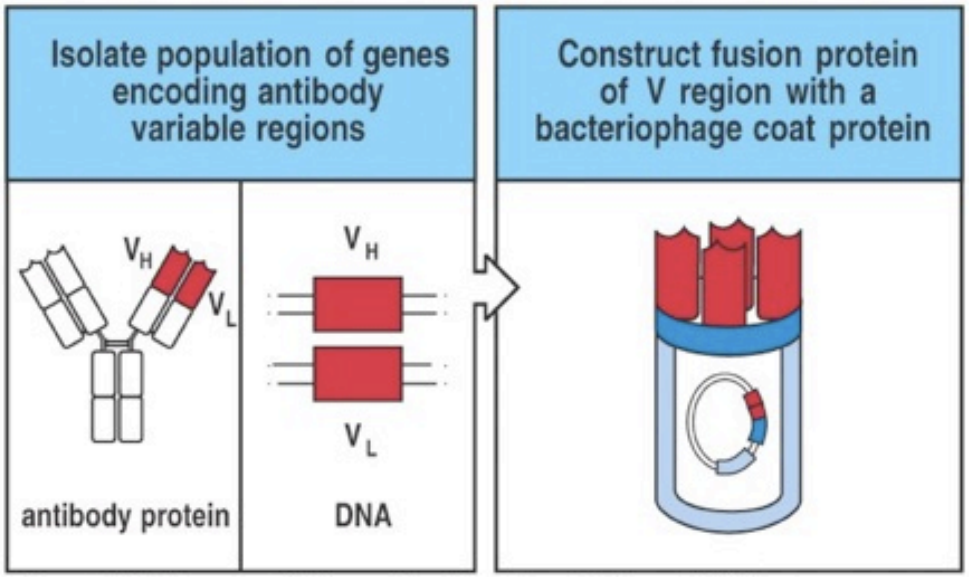




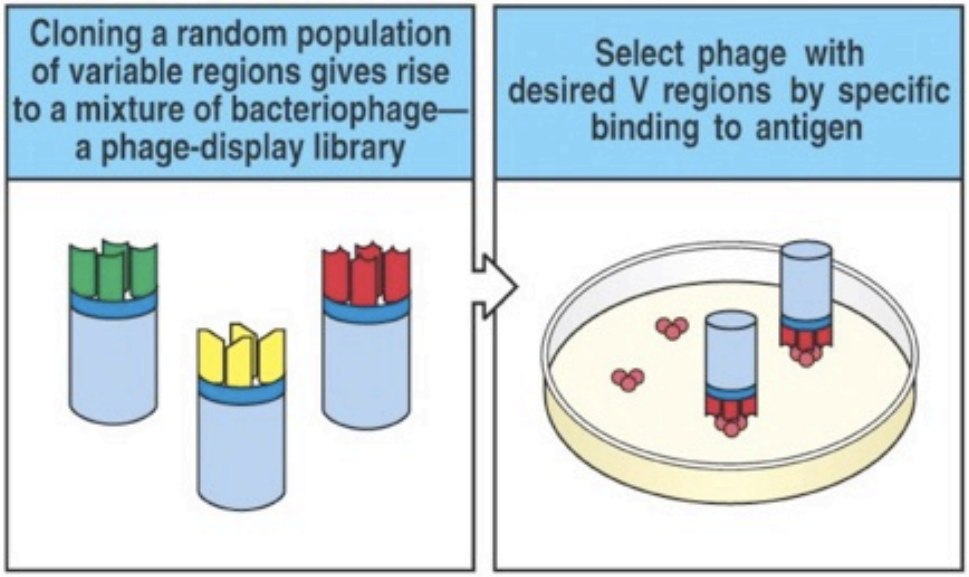
Antibody engineering

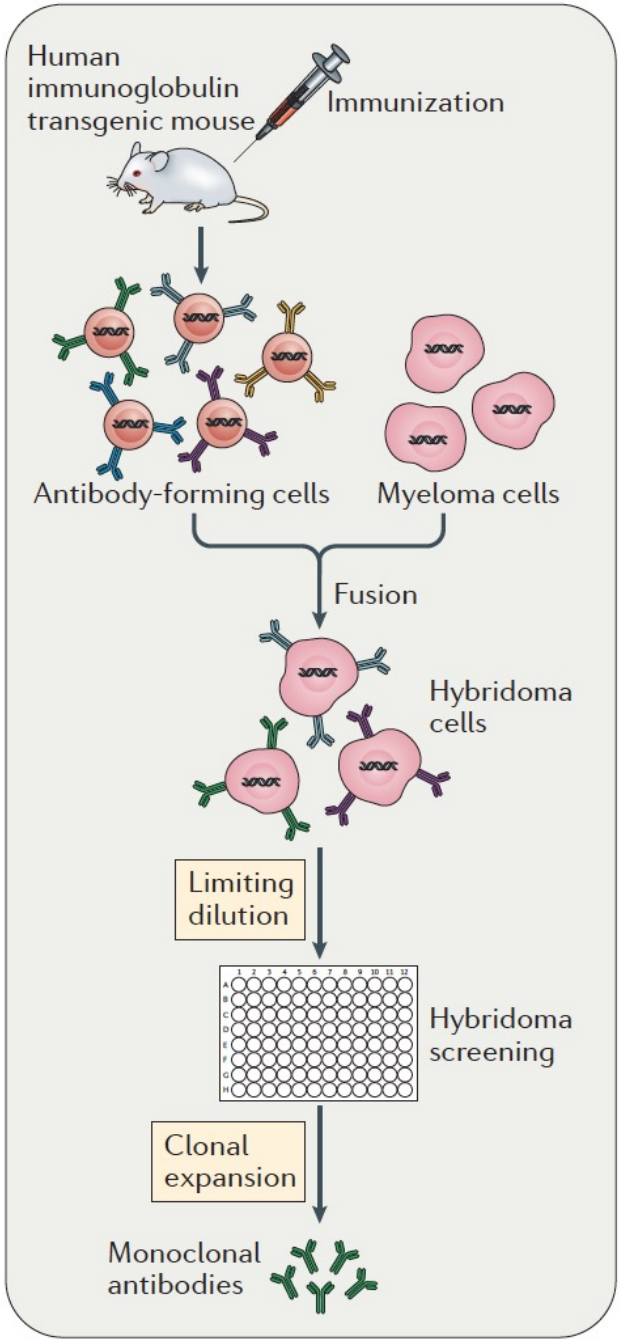


In vitro and in vivo human antibody techniques: phage display and transgenic mouse technologies



human antibody library





Evolution of therapeutic antibody technologies and progress to the clinic

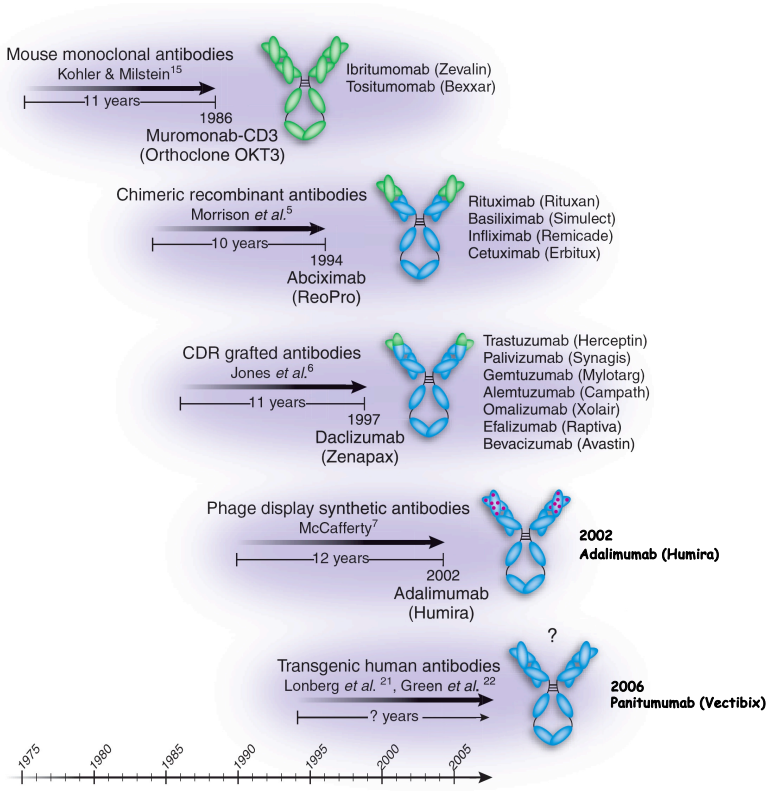
1. Murine mAbs

Genetic manipulation of murine mAbs:

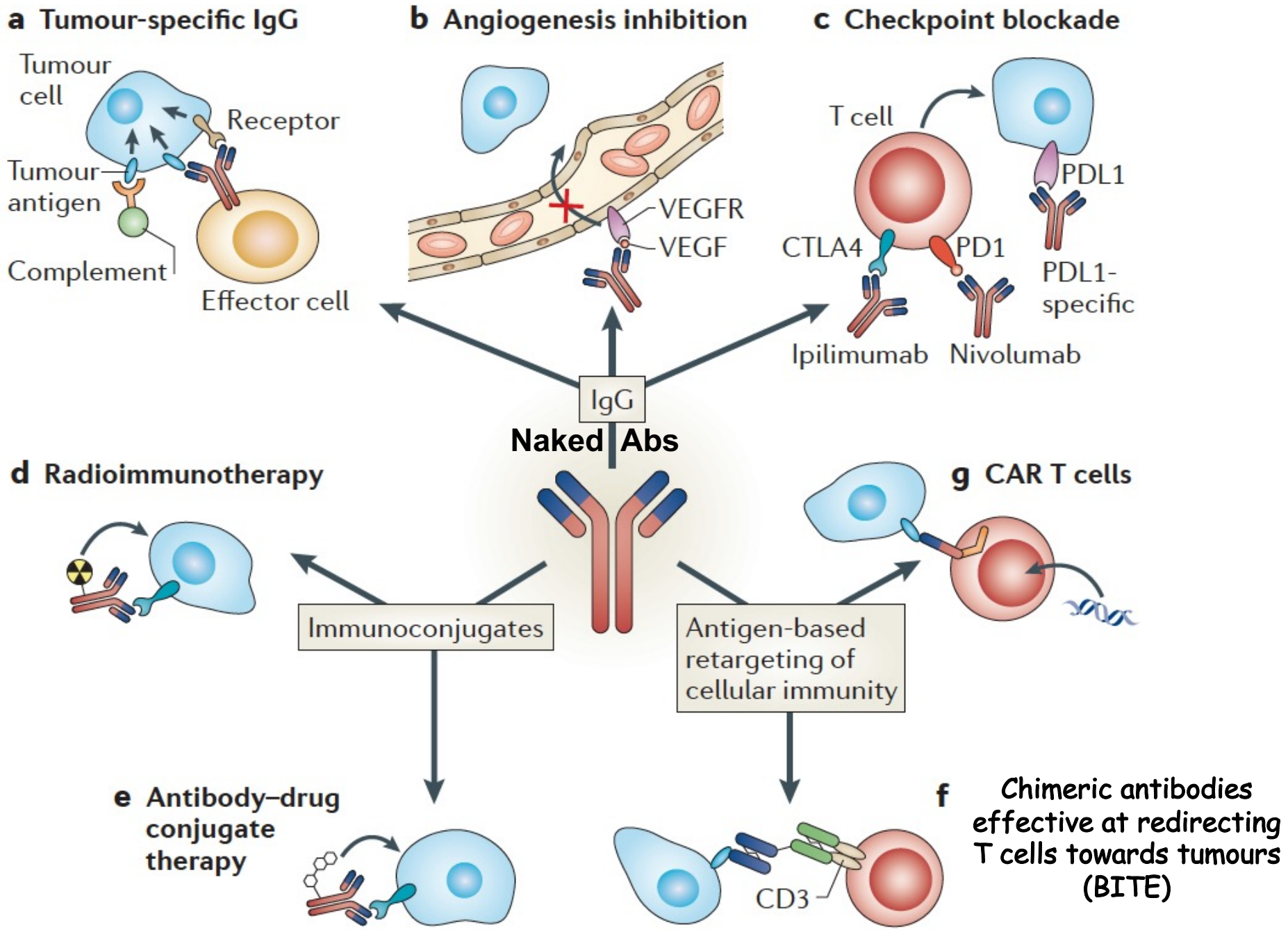
- Chimeric mAbs (V murine, C human)
- Humanized mAbs (mouse CDR only)

2. Human Mabs

- Phage display
- Transgenic mice



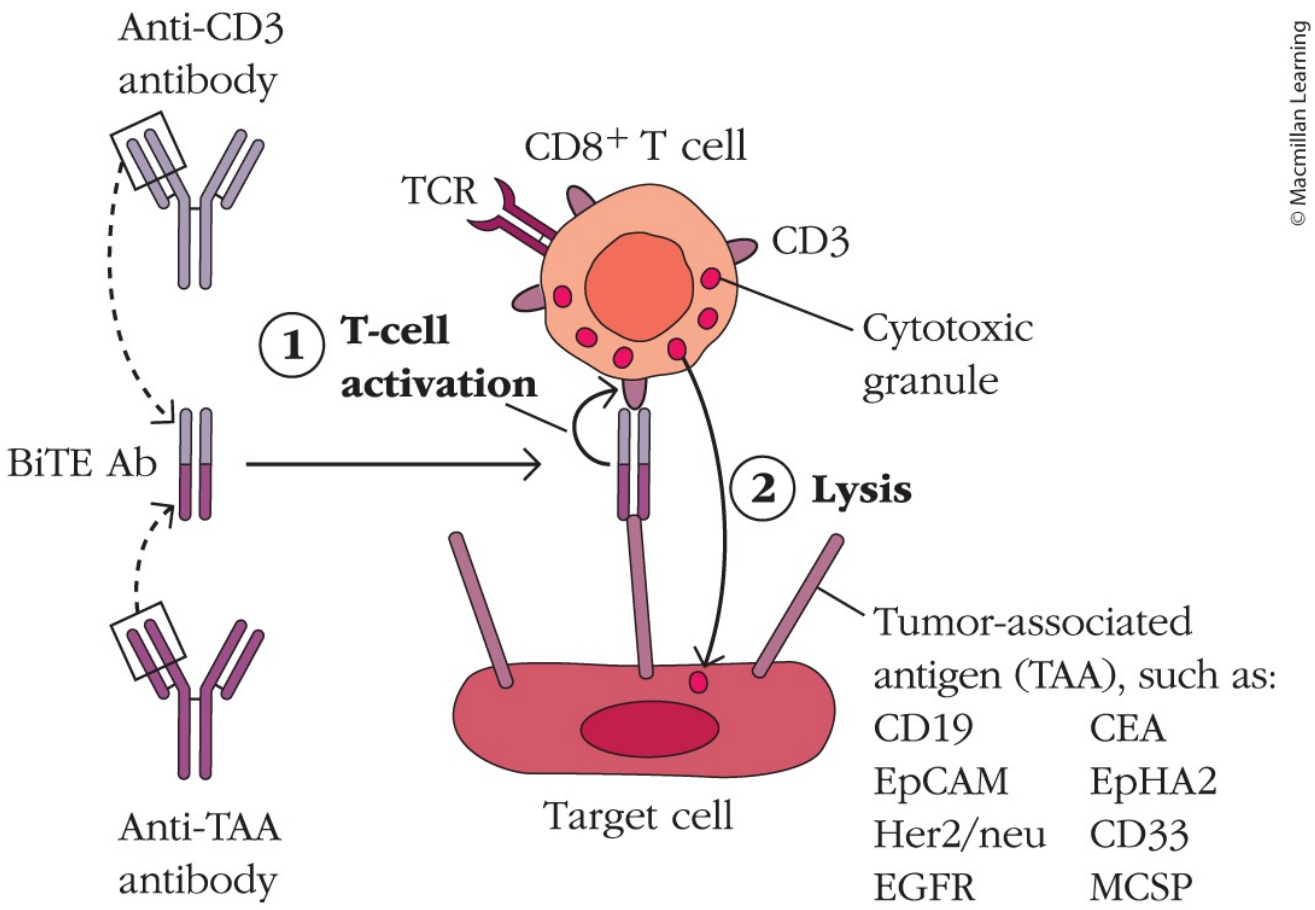
Monoclonal Antibody-based therapeutic strategies



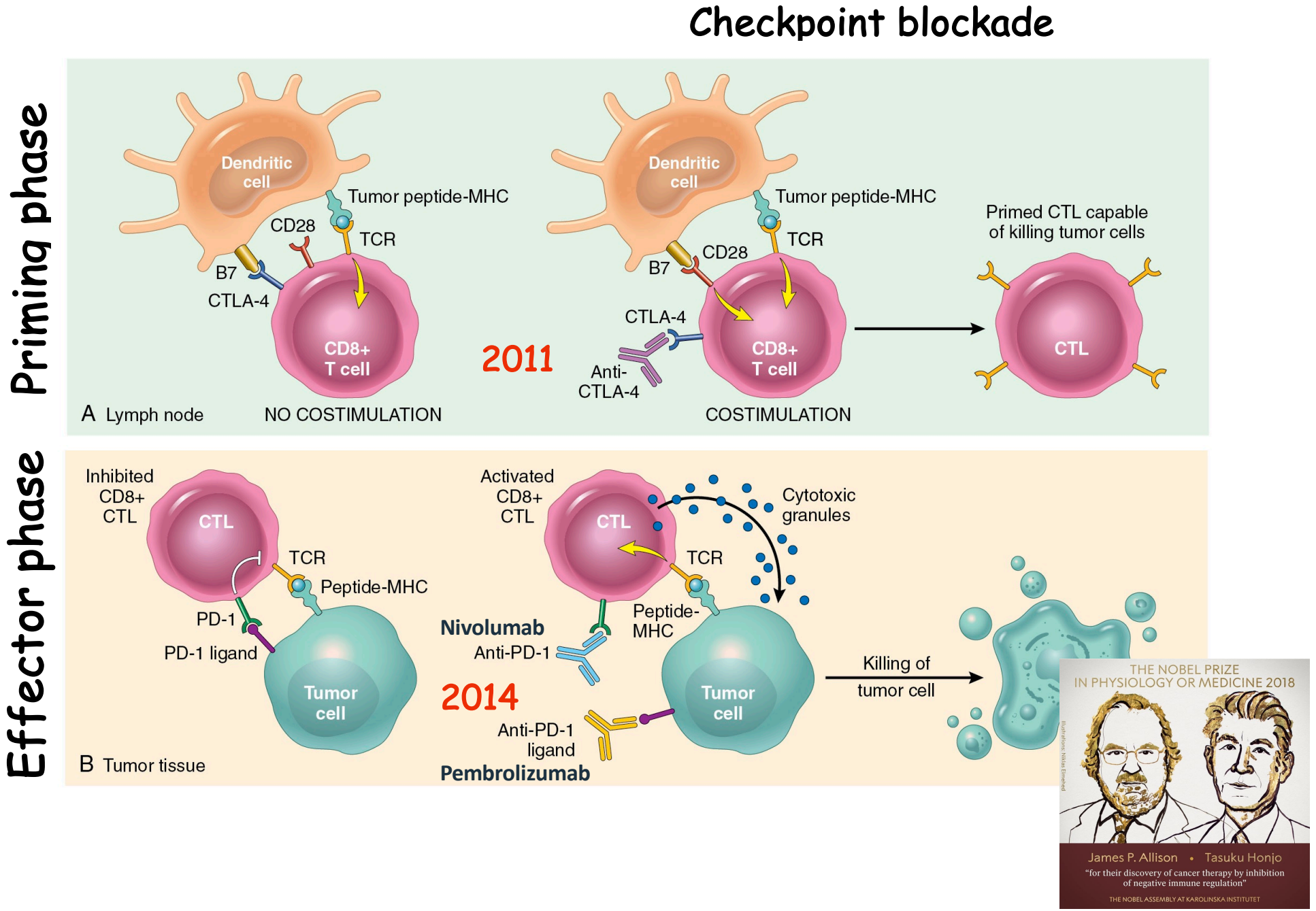
Chimeric antibodies effective at redirecting T cells towards tumours (BITE)

Bispecific T-cell engagers (BiTEs) used in cancer immunotherapy

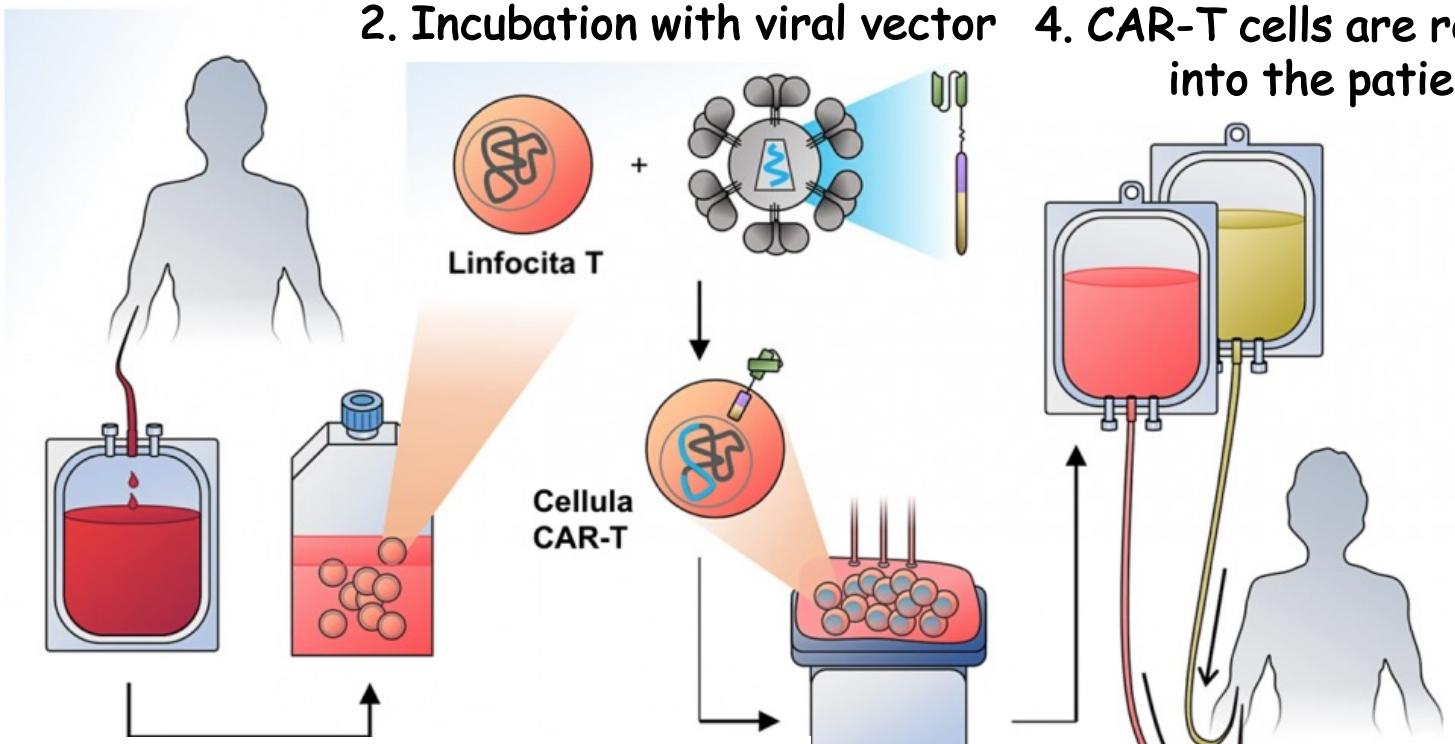
- BiTES are made by combining light- and heavy-chain variable regions of two antibodies into a single chain
- recognize two target epitopes
- activate T cells



Checkpoint blockade for cancer immunotherapy



Genetically modified T lymphocytes for cancer therapy (CAR-T)



CAR-T (Chimeric Antigen Receptor- T)

August 2017

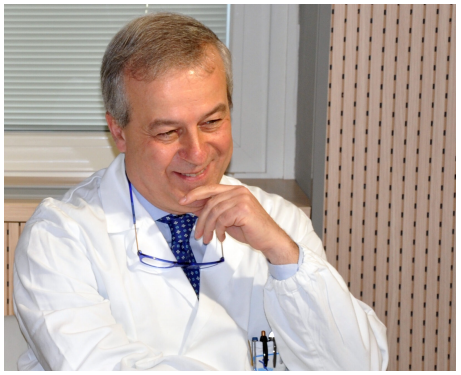
FDA approves first CAR T therapy

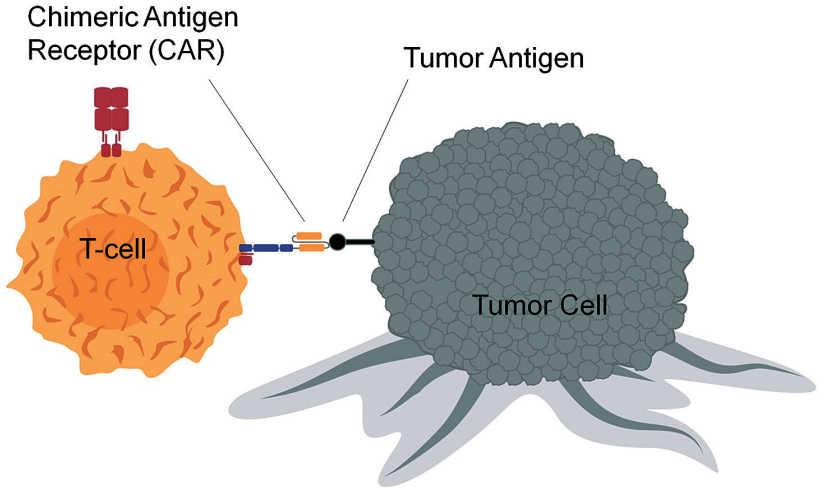
The FDA gave the green light to Novartis's tisagenlecleucel for the treatment of acute lymphoblastic leukaemia (ALL), marking a historic approval for a first-in-modality chimeric antigen receptor (CAR) T cell therapy.

October 2017

Second anticancer CAR T therapy receives FDA approval

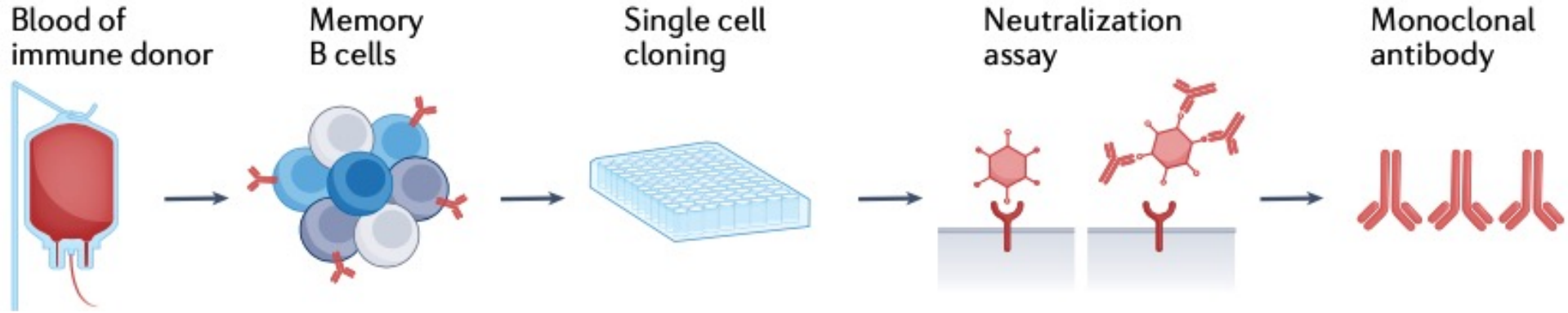
The FDA's approval of Gilead Sciences and Kite Pharmaceutical's axicabtagene ciloleucel for relapsed or refractory large B cell lymphoma marked the second green light for the emerging chimeric antigen receptor (CAR) T cell modality.





Human monoclonal neutralizing antibodies : a novel agnostic approach

Only a very small proportion of infected individuals generates broadly and potent neutralizing antibody



Super-antibodies offer a new promise for prophylaxis and therapy of infections for viruses that are highly antigenically variable, are newly emerging or have pandemic potential

The NEW ENGLAND JOURNAL of MEDICINE

Monoclonal Antibodies for Emerging Infectious Diseases — Borrowing from History

Hilary D. Marston, M.D., M.P.H., Catharine I. Paules, M.D., and Anthony S. Fauci, M.D.

Antiviral super-antibodies are now available against:

HIV, Influenza virus, RSV, HCMV, Rabies virus,
Dengue and Zika virus, Ebola virus, SARS