

Monoclonal antibody: an overview

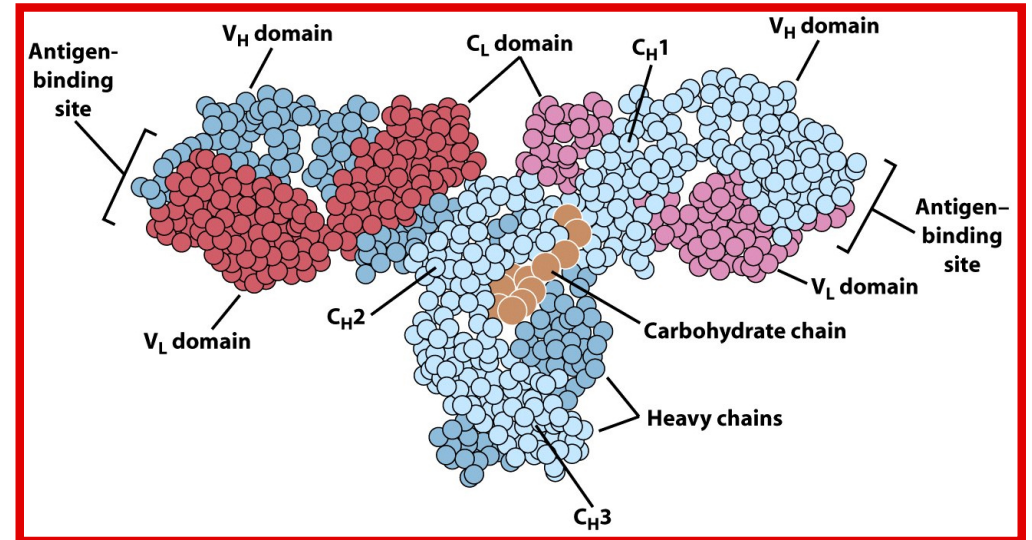
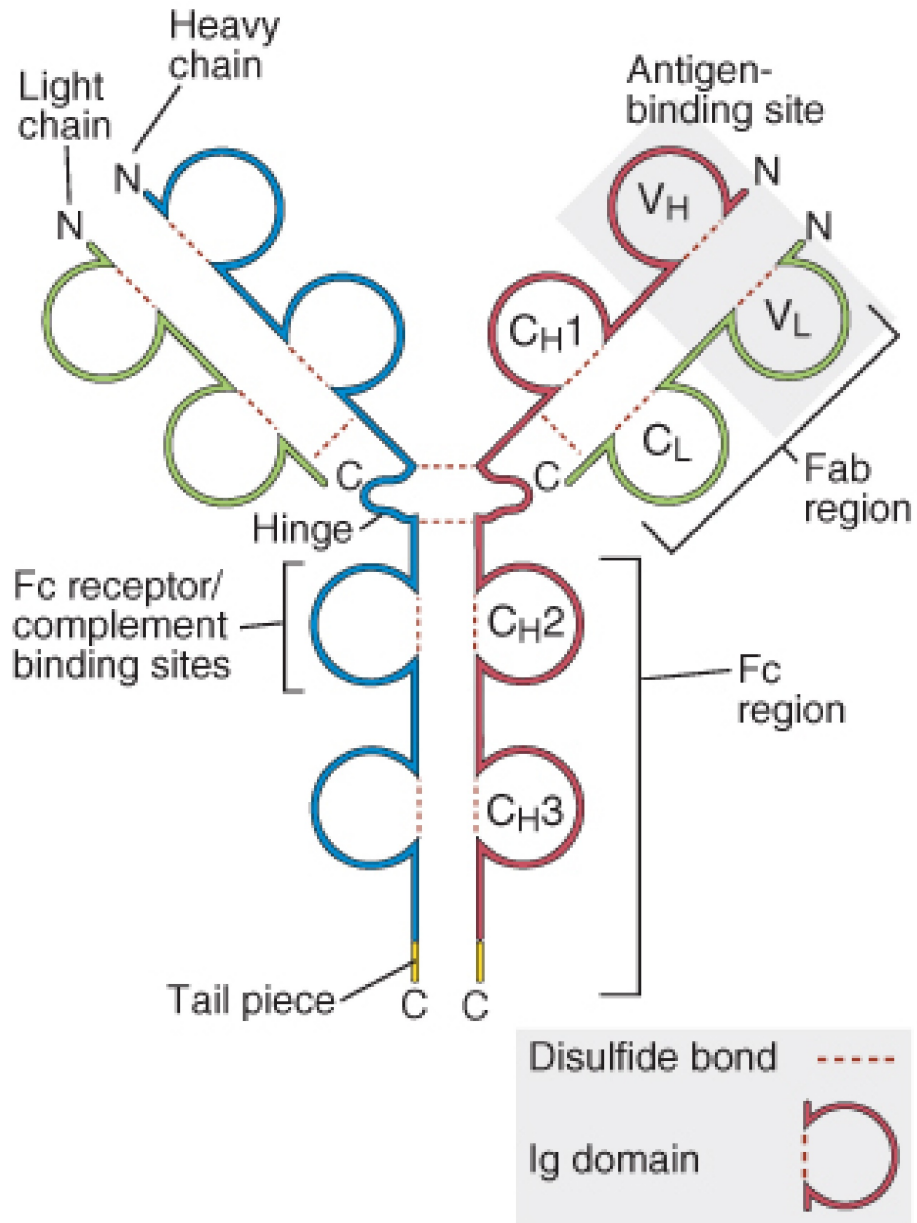
Prof. Ricciarda Galandrini



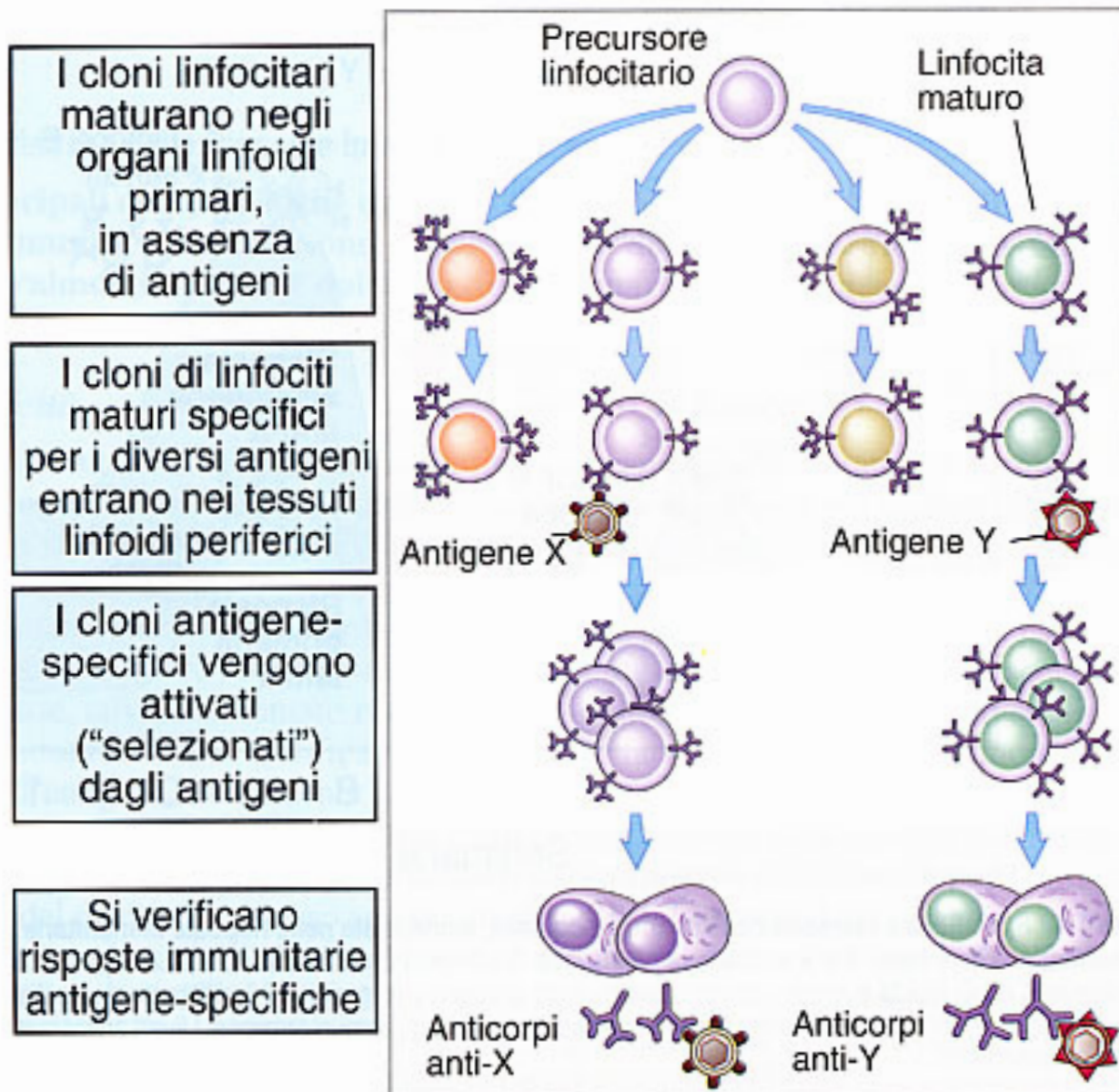
SAPIENZA
UNIVERSITÀ DI ROMA

Anticorpo o immunoglobulina

(A) Secreted IgG



PRODUZIONE CLONALE DEGLI ANTICORPI



LA RISPOSTA ANTICORPALE *IN VIVO* E' DI TIPO POLICLONALE

Antigene

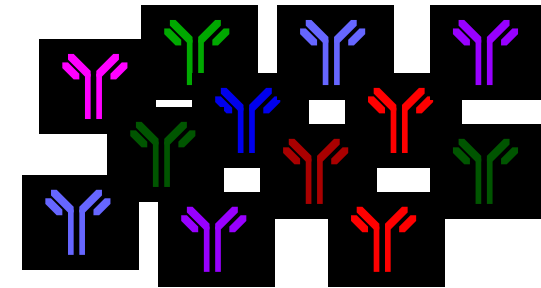


Tanti epitopi diversi

Immunizzazione



Anticorpi



Si ottiene una miscela di anticorpi, prodotti dai diversi cloni di linfociti B, capaci di legarsi a tutti gli epitopi dell'antigene con specificità e affinità diverse.



Antisiero o Anticorpo policlonale

ANTICORPI MONOCLONALI (mAb)

- **Specificità definita e omogenea**
- **Isotipo omogeneo**
- **Elevata affinità**
- **Produzione in grande quantità**
- **Disponibilità illimitata**
- **Elevata purezza**

Kohler 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**"

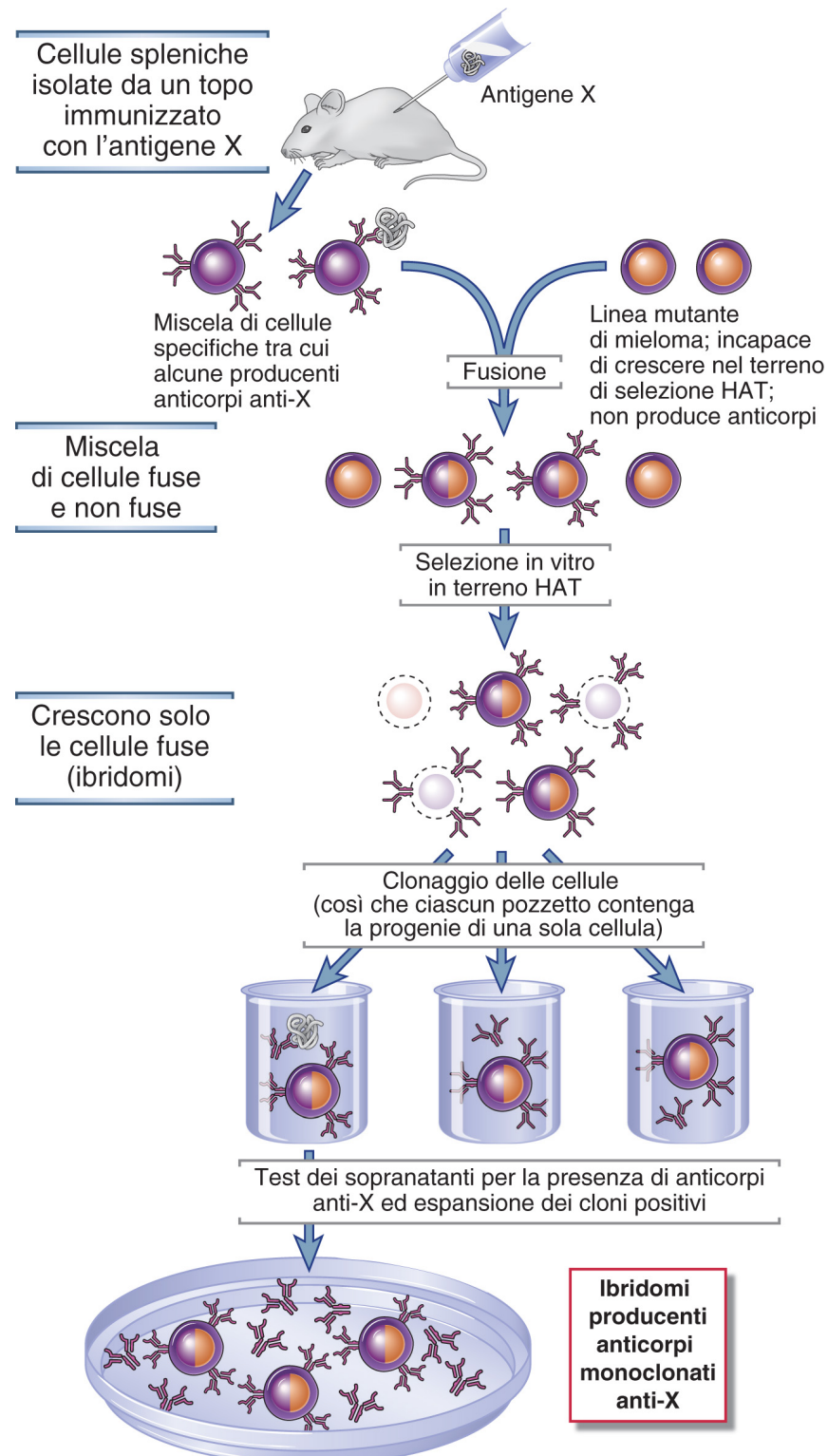


Georges J. F. Köhler

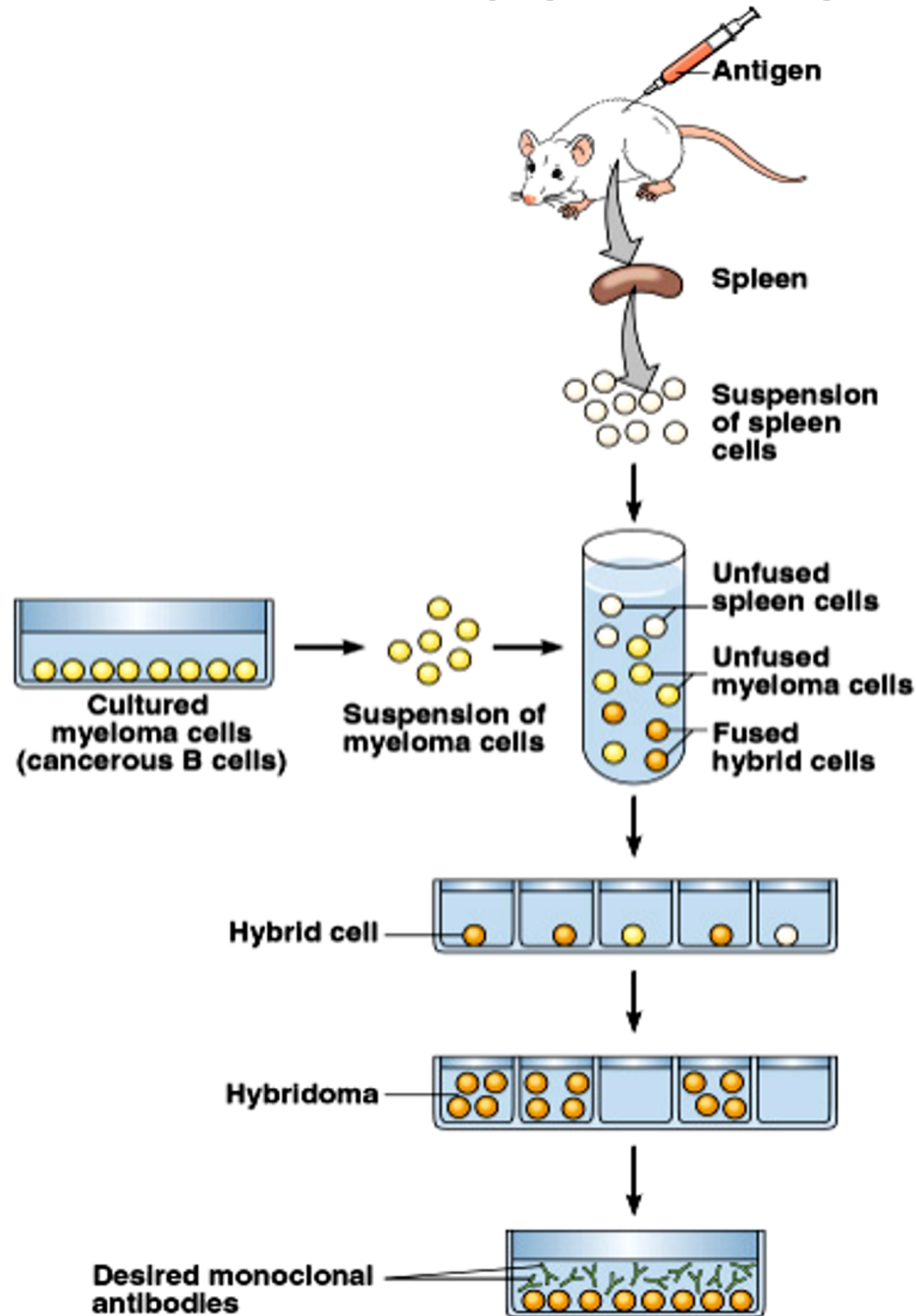


César Milstein

Produzione di anticorpi monoclonali



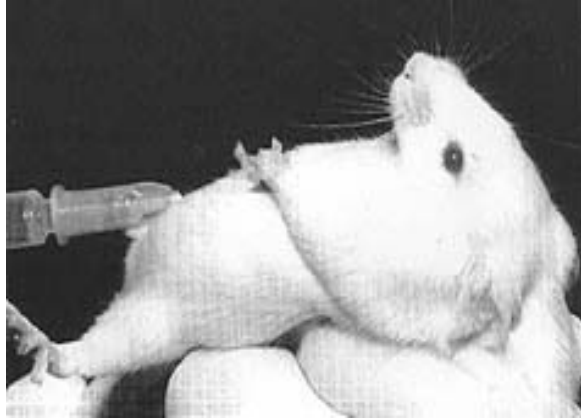
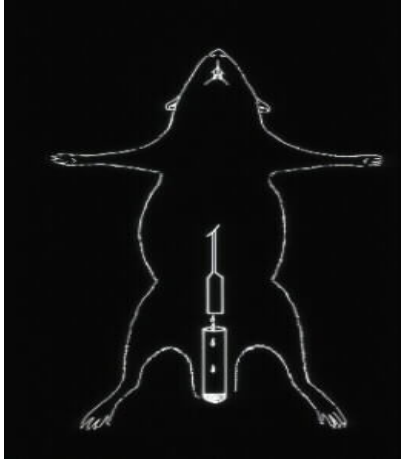
ANTICORPI MONOCLONALI



i sopranatanti di coltura vengono sottoposti a screening: ELISA, citofluorimetria, Western Blot

selezioni di cloni ad elevata affinità

PRODUZIONE DI ANTICORPI MONOCLONALI



Generazione di ascite

(5-20 mg/ml)



Biofermentatori

(5 mg/ml)

*purificazione mediante
cromatografia di affinità*

Monoclonal antibodies:

the story of a discovery that revolutionized science and medicine

APPLICAZIONI DEGLI ANTICORPI MONOCLONALI

In vitro

- **Identificazione di popolazioni cellulari esprimenti marcatori specifici**

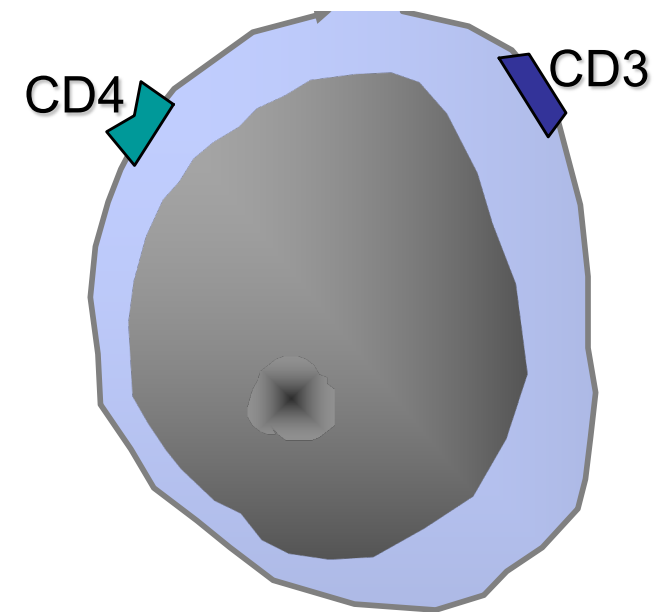
}	immunoistochimica
	immunocitochimica
	immunofluorescenza
	immunofenotipo
- **Dosaggio di molecole secrete**

}	ELISA, RIA

In vivo

- **Diagnostica per immagine**
- **Immunoterapia (target therapy)**
 - **Malattie oncologiche**
 - **Malattie autoimmuni**
 - **Malattie cardiovascolari**

Immunofenotipo leucocitario



- L'immunofenotipizzazione è basata sull'identificazione di antigeni di superficie (ma anche citoplasmatici o nucleari), per mezzo di anticorpi monoclonali coniugati con fluorocromi. La presenza di un dato antigene è, infatti, un indicatore dell'appartenenza di una cellula ad uno stipe e/o ad un definito stadio differenziativo o funzionale.
- Mediante immunofenotipizzazione è possibile quantificare diverse popolazioni cellulari.

PERCENTUALE DI LEUCOCITI NEL SANGUE

-Neutrofili	60-70
-Eosinofili	2-5
-Basofili	< 1
-Monociti	3-7
-Linfociti	20-30

LINFOCITI T

CD3 58-88%

LINFOCITI B

2-19 %













CELLULE NK

2-28%

Le cellule emopoietiche sono identificabili mediante anticorpi monoclonali: marcatori di popolazione

CD:
cluster of differentiation

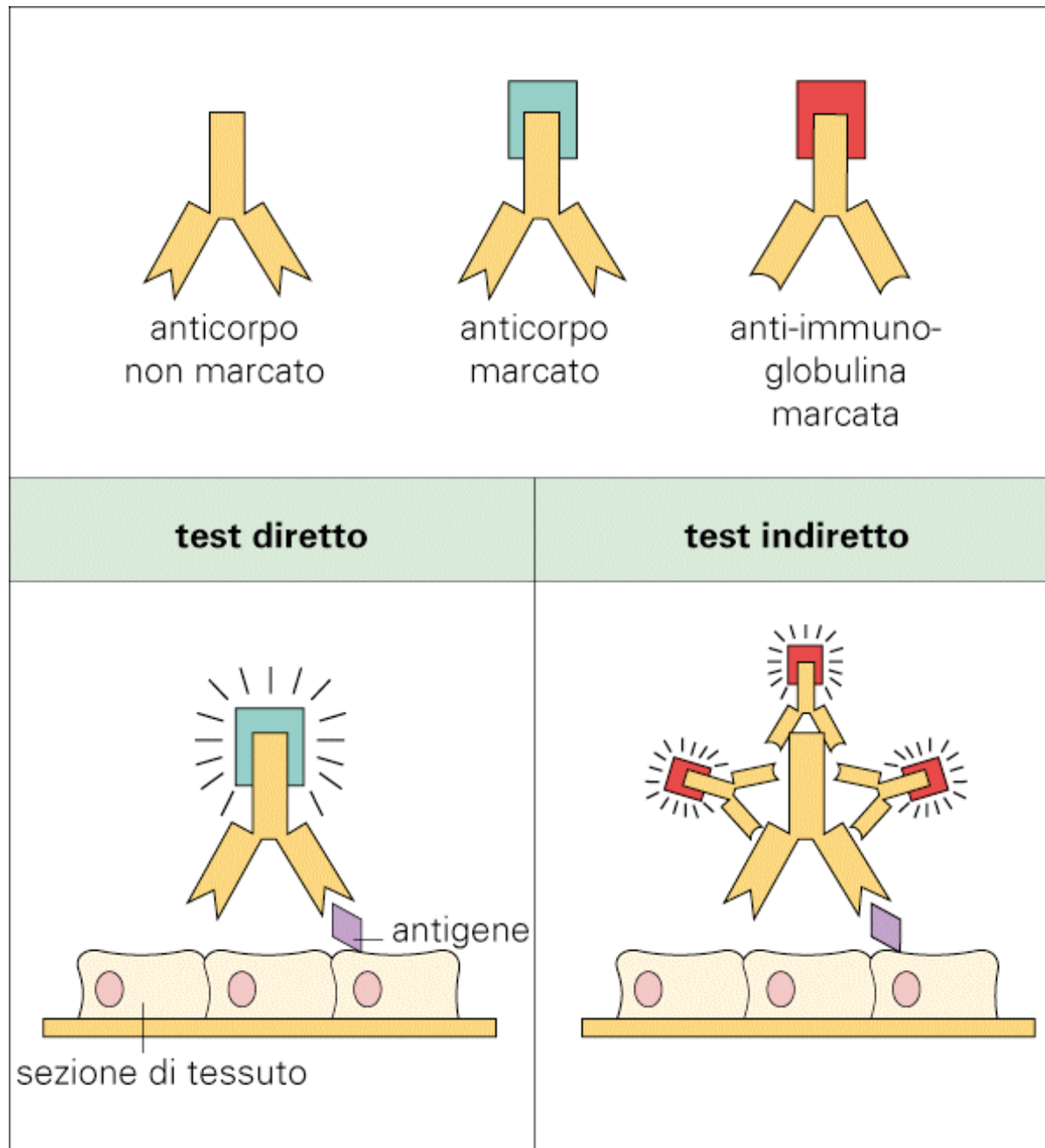
CD antigens by differentiation cell

		Key Antigens-Human			Key Antigens-Human
T Cell		CD3 CD4 CD8	Monocyte		CD33
B Cell		CD19 CD20	Granulocyte		CD66b
Dendritic Cell		CD11c CD123	Platelet		CD41 CD61 CD62
NK Cell		CD56	Erythrocyte		CD235a
Stem Cell		CD34	Endothelial		CD146
Macrophage		CD14	Epithelial		CD326

PANNELLO DI ANTICORPI MONOCLONALI UTILIZZATI PER L'ANALISI DEL FENOTIPO DI LEUCOCITI DI SANGUE PERIFERICO

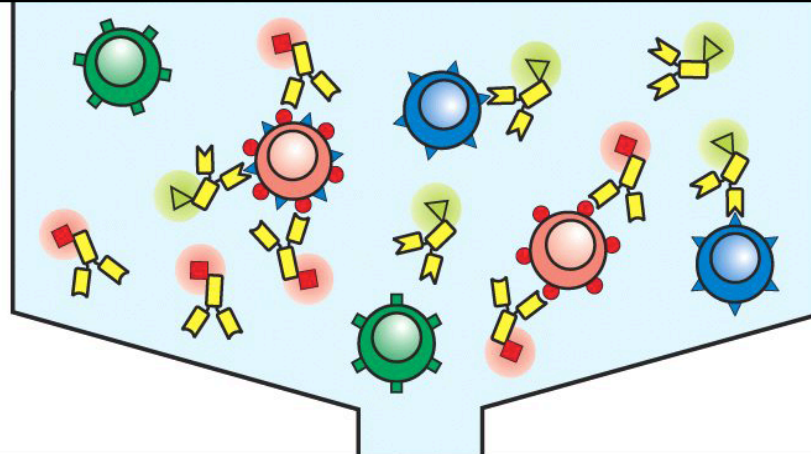
Anti-CD45	leucociti
Anti-CD3	
Anti-CD4	linfociti T
Anti-CD8	
Anti-CD19	linfociti B
Anti-CD56	
Anti-CD16	cellule NK
Anti-CD14	monociti

Immunofluorescenza



Il citometro a flusso

1 Una miscela di cellule è marcata con anticorpi fluorescenti



2

Le cellule vengono fatte passare attraverso un foro formando un flusso continuo che passa attraverso un raggio laser

3

Un tubo fotomultimetro capta la luce diffusa da ogni singola cellula dando un'idea della grandezza e della granulosita' della cellula stessa, e l'emissione dei diversi coloranti fluorescenti

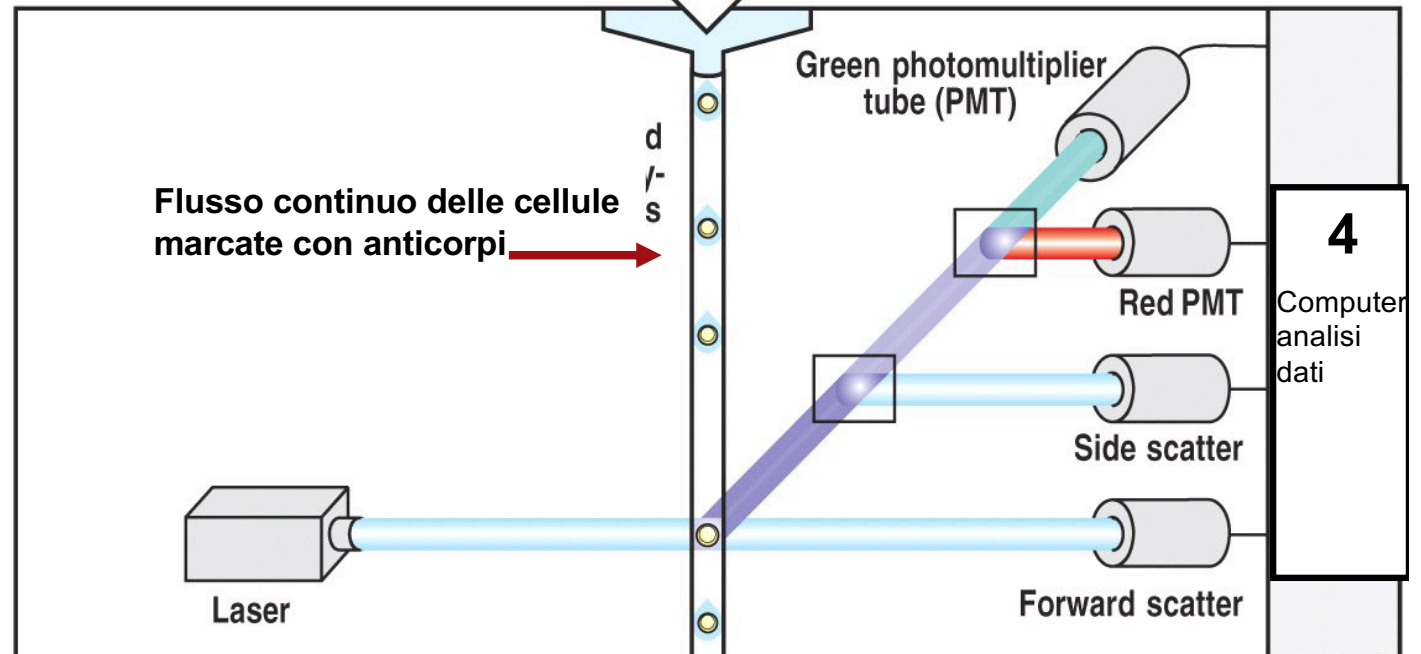
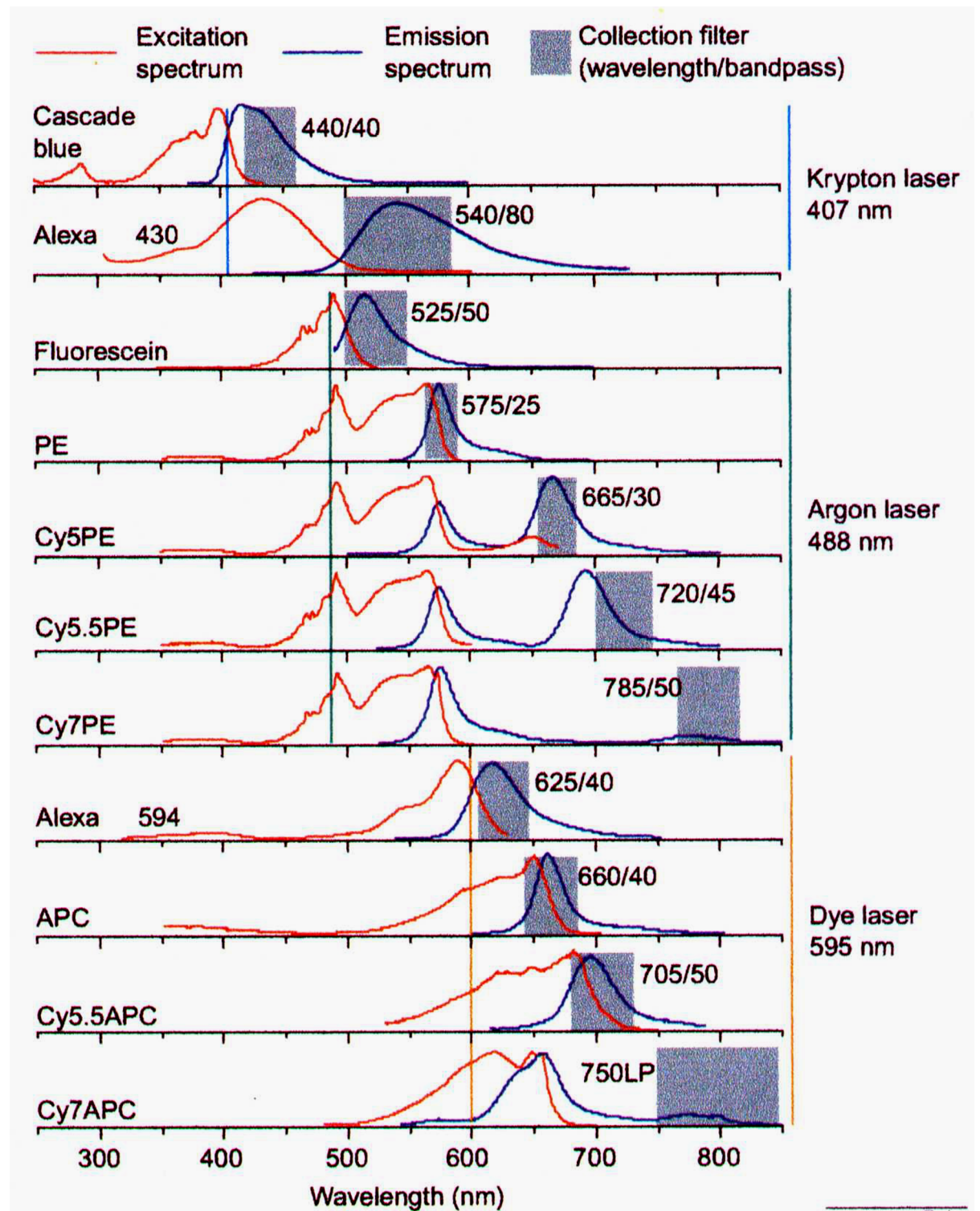
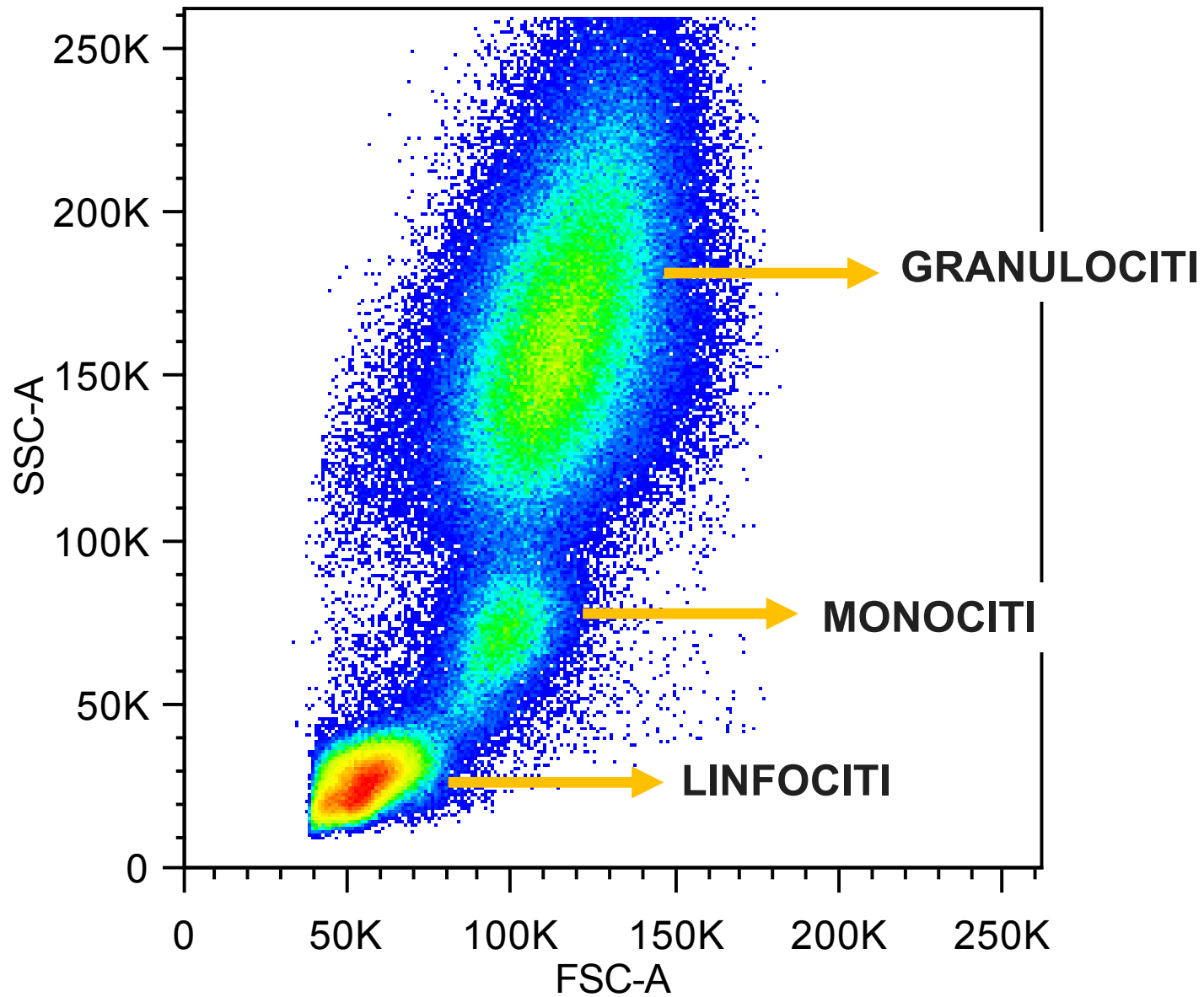


Figure A-25 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Fluorocromi

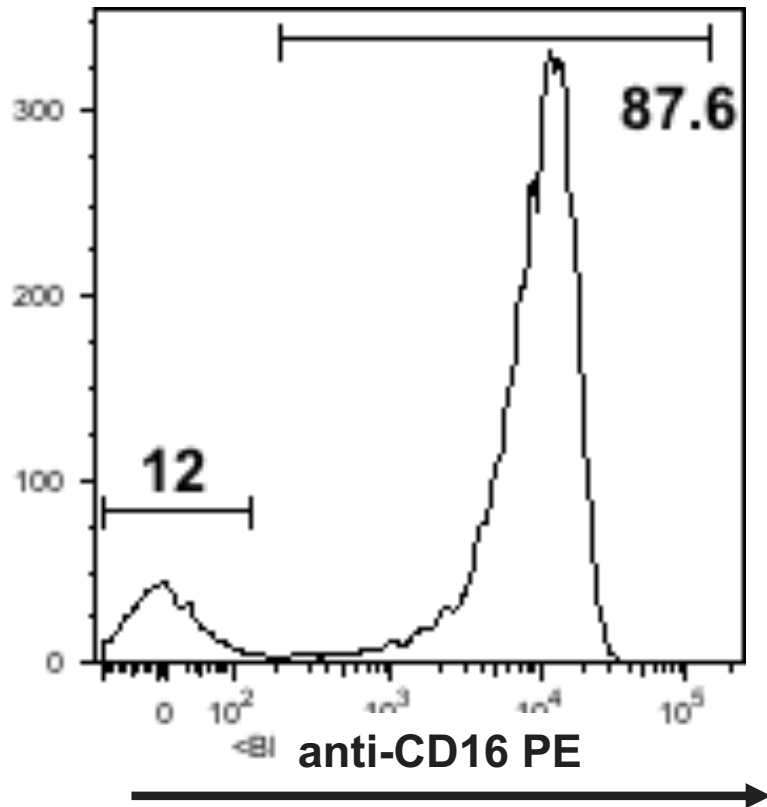


**“GATING” sui PARAMETRI FISICI
FSC=DIMENSIONE SSC=GRANULOSITA’**

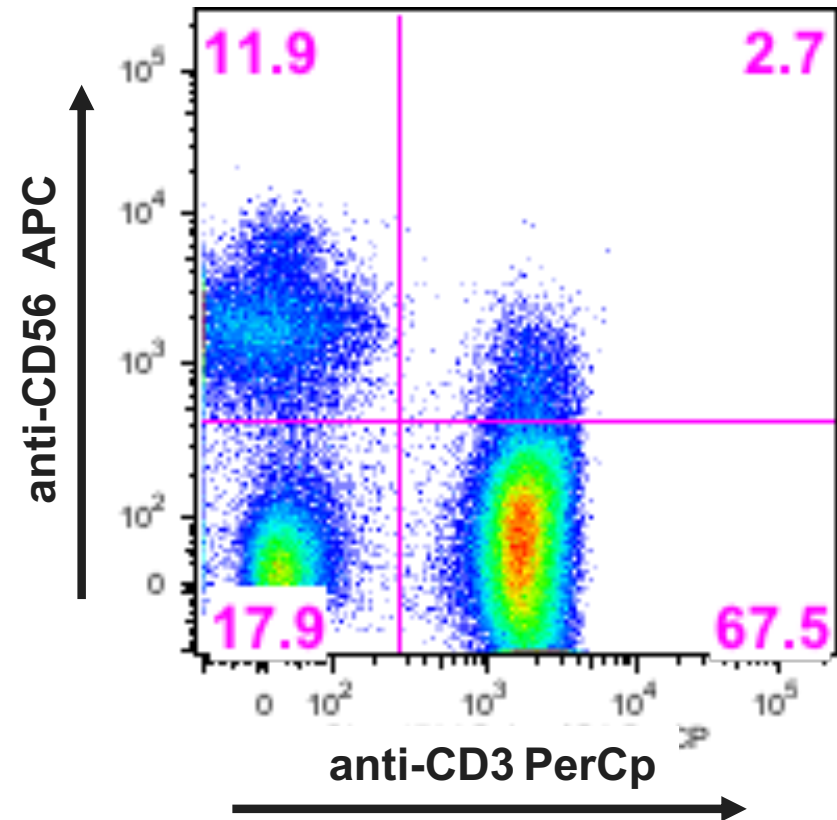


RAPPRESENTAZIONE DELLA FLUORESCENZA: ISTOGRAMMA AD UN PARAMETRO E A DUE PARAMETRI

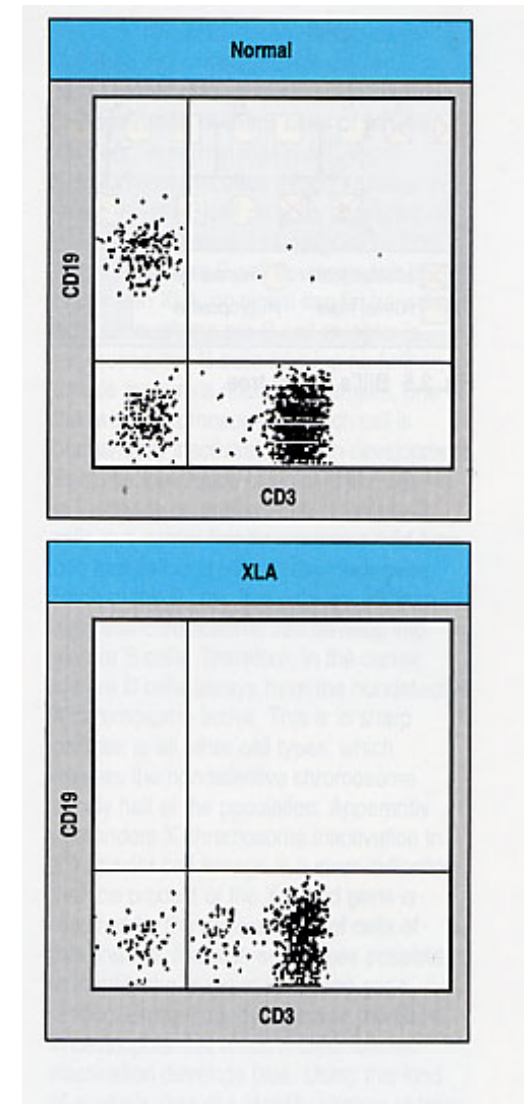
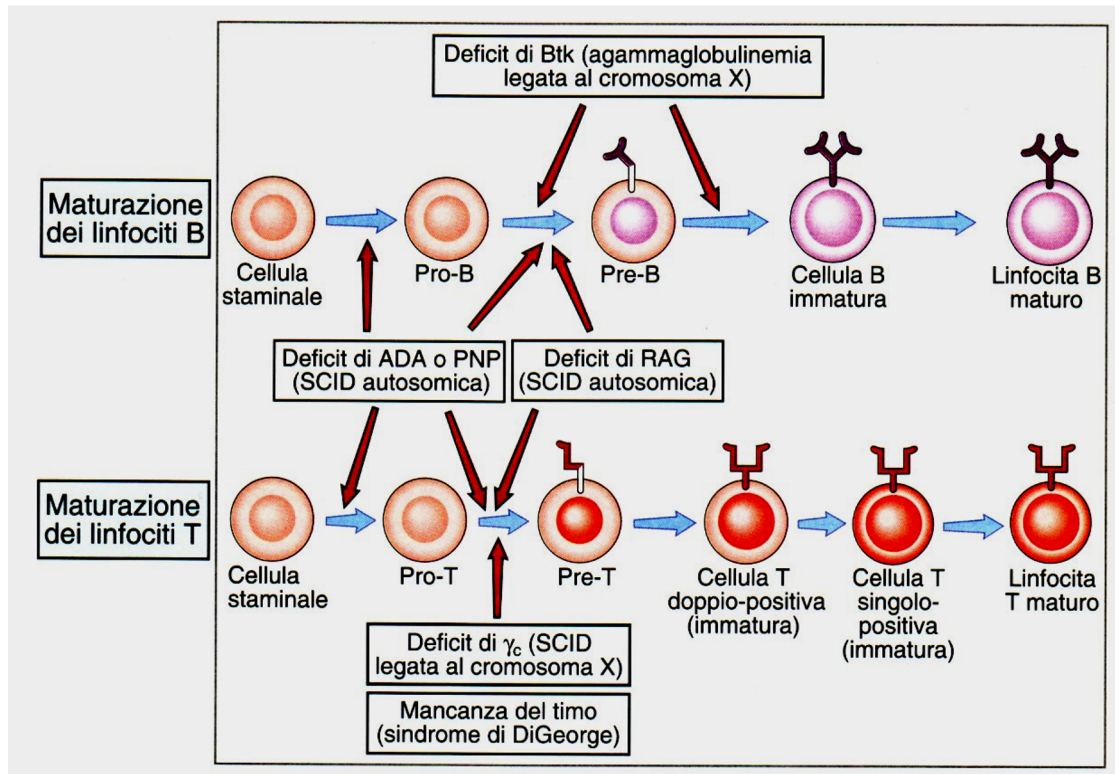
ANALISI
SINGOLO PARAMETRO



ANALISI
MULTIPARAMETRICA



Agammaglobulinemia legata al cromosoma X (malattia di Bruton)



Espressione di marcatori tipici per leucemie e linfomi

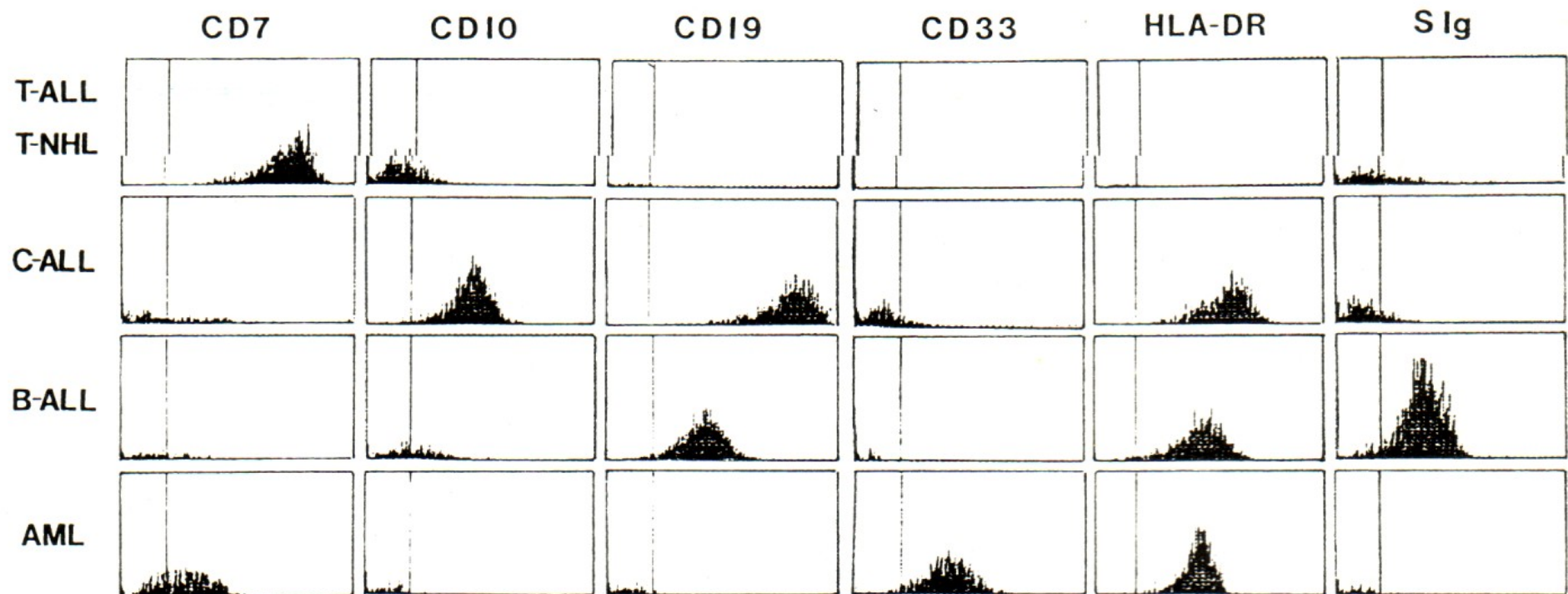
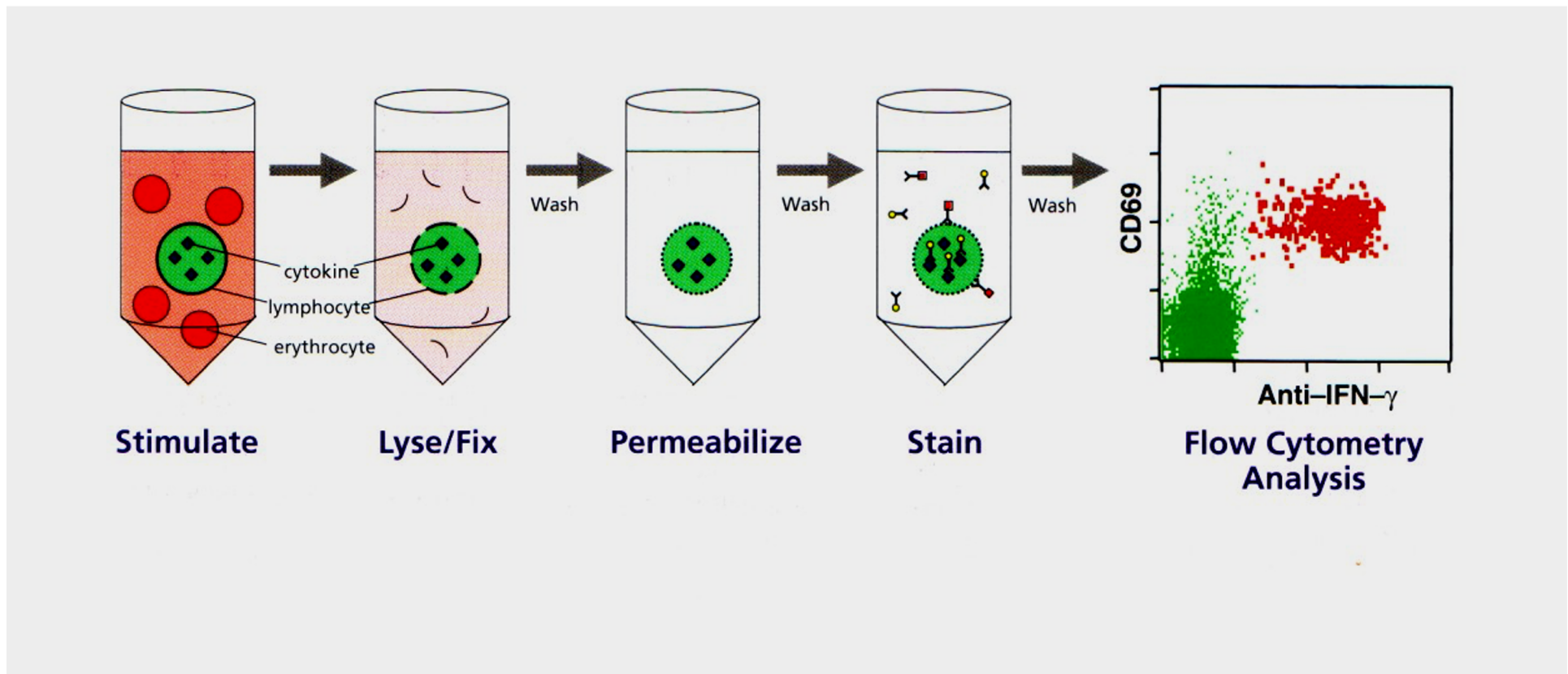


Figura 1: Analisi citofluorimetrica di leucemie e linfomi marcati con anticorpi monoclonali.

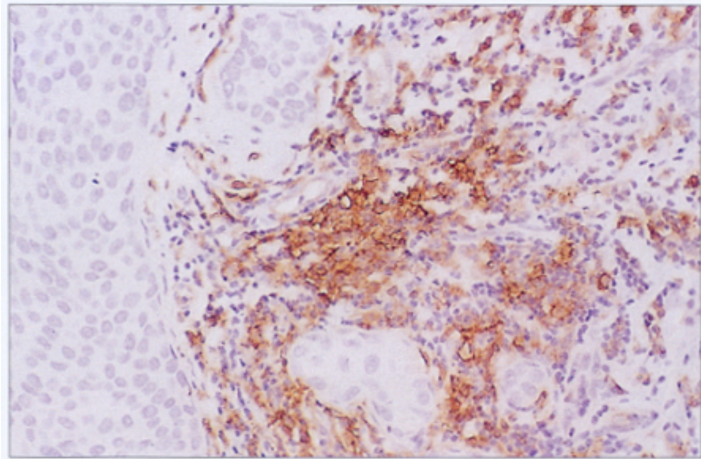
Analisi funzionale: produzione di citochine



Stimolazione, Permeabilizzazione, Colorazione per l'analisi delle citochine intracellulari

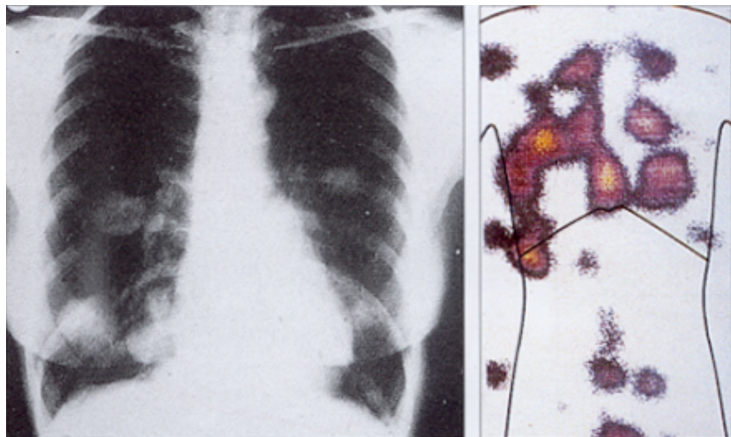
DIAGNOSTICA PER IMMAGINI

in vitro



**Immunoistochimica
mAb anti-CEA**

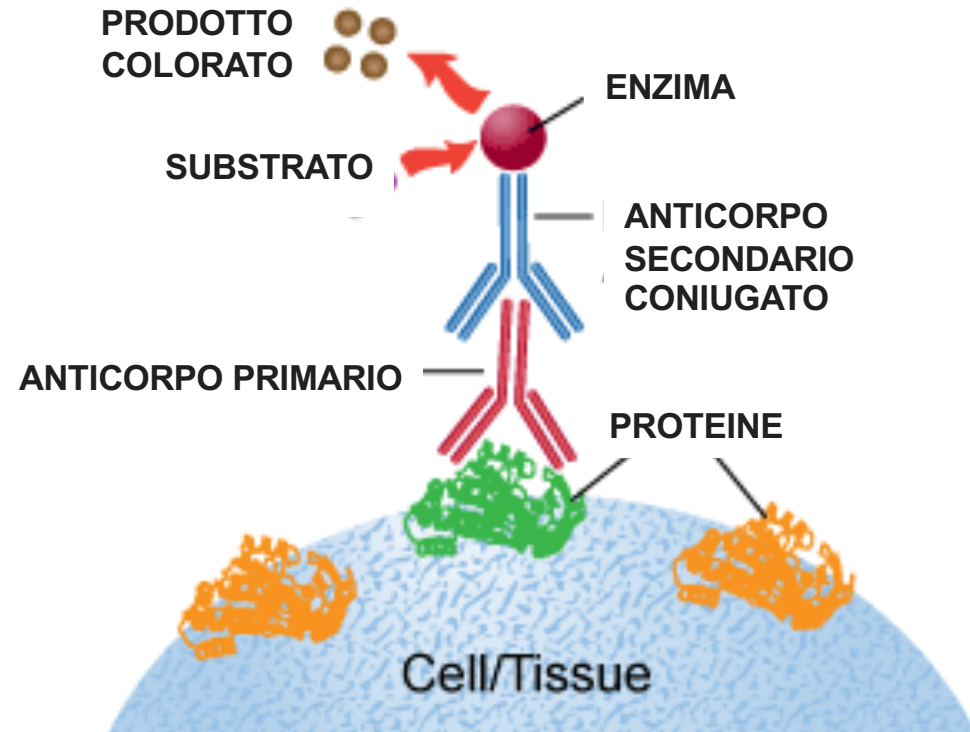
in vivo



^{99m}Tc-mAb anti-CEA

Technetium-99m (^{99m}Tc) isomero metastabile del tecnezio che decade in 6 ore. Beta emittente . 99Tc decade in 211 anni.

Indirect Immunohistochemistry



ENZIMA

❖ PEROSSIDASI DI RAFANO

❖ FOSFATASI ALCALINA



SUBSTRATO

DIAMMINOBENZIDINA

BCIP/NBT

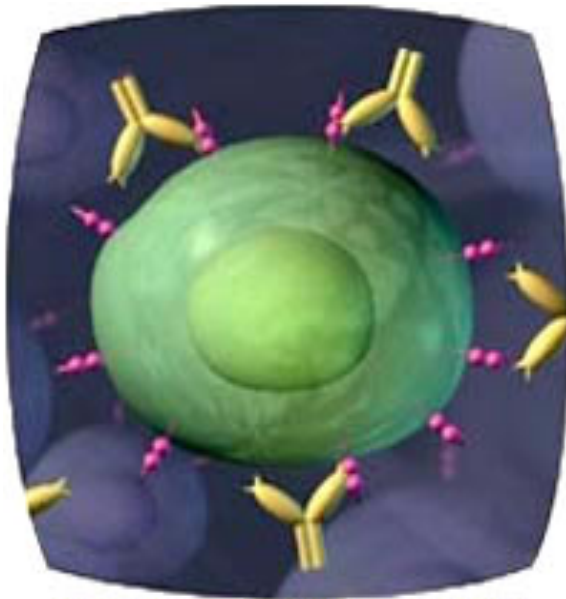


Utilizzo dei mAb in campo terapeutico

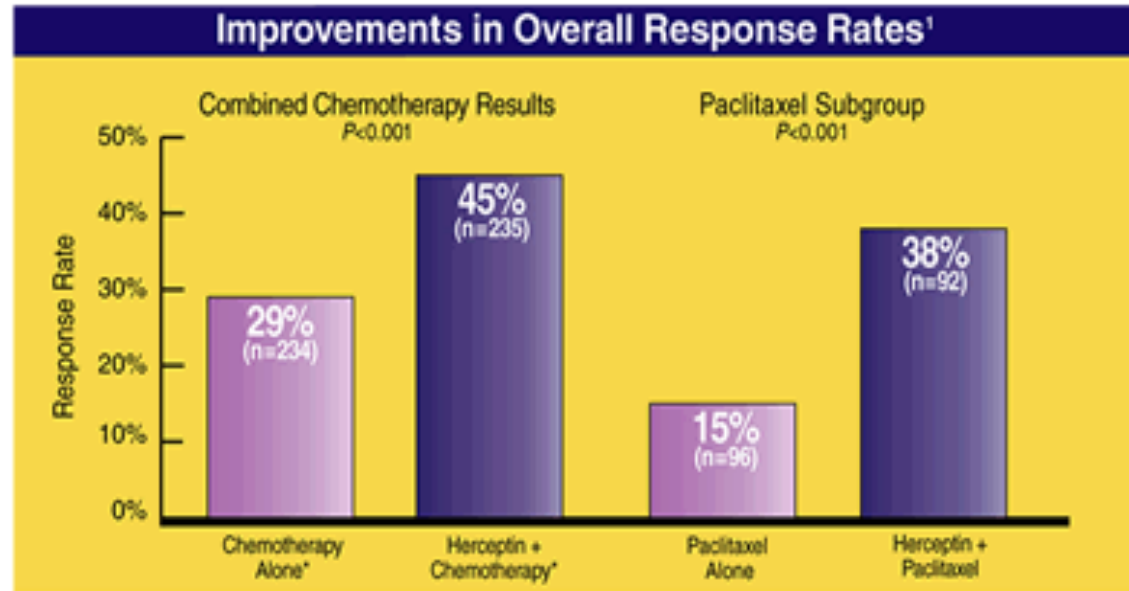
- Tumori
- Rigetto di trapianti
- Malattie cardiovascolari
- Malattie infettive
- Malattie autoimmuni

TUMOR TARGETING mAbs

Trastuzumab: la storia di un successo



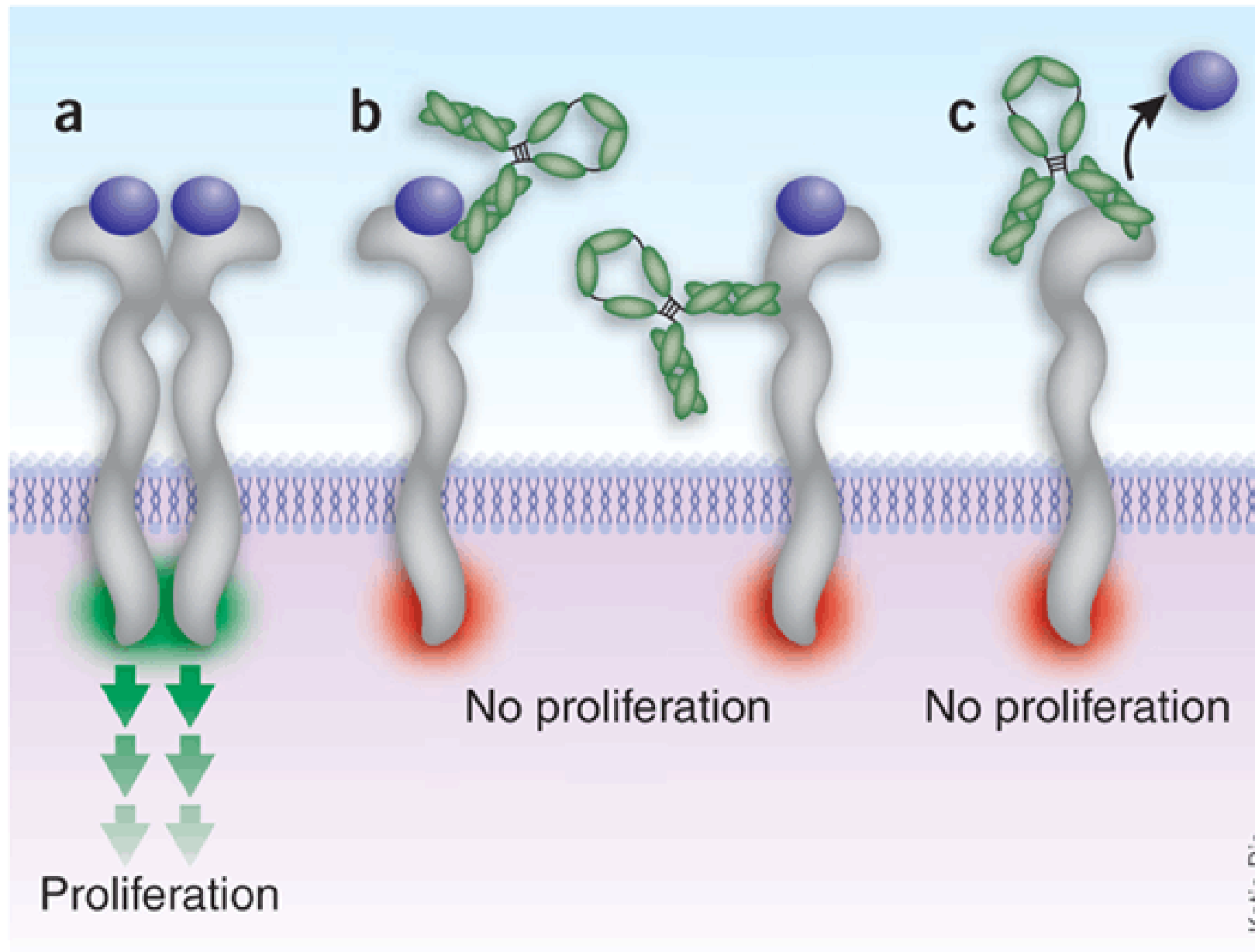
Monoclonal antibodies targeting a HER2 protein overexpressing cell



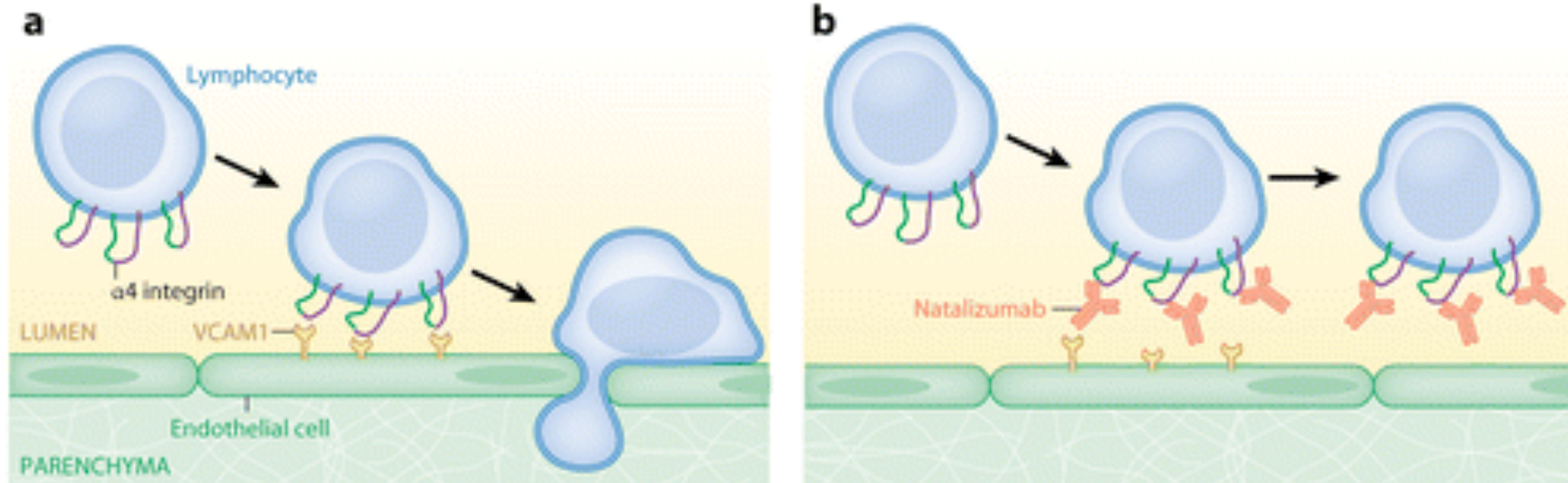
*Chemotherapy = either doxorubicin or epirubicin plus cyclophosphamide, or paclitaxel.

Come si esplica l'effetto antitumorale?

**GLI ANTICORPI MONOCLONALI BLOCCANO SELETTIVAMENTE
LA PROLIFERAZIONE DELLE CELLULE TUMORALI: effetti diretti**



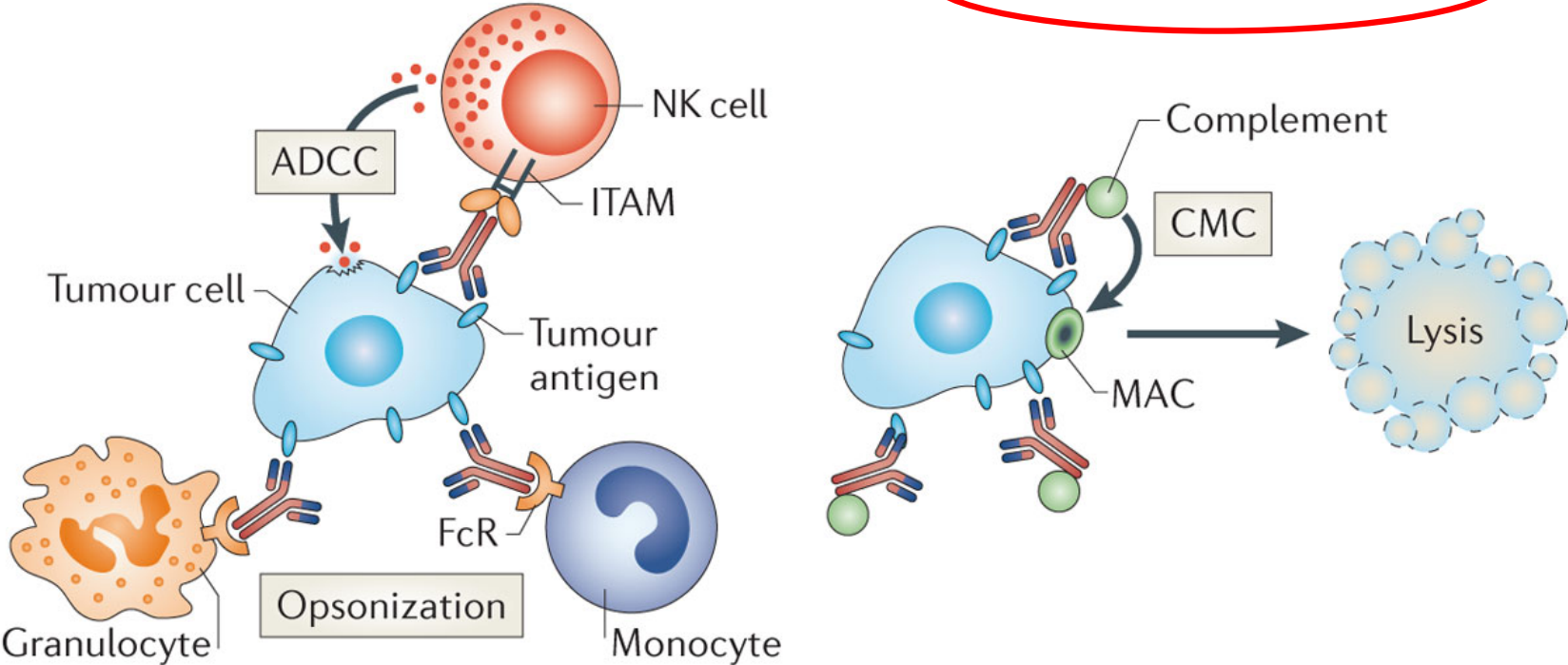
The discovery of natalizumab, a potent therapeutic for multiple sclerosis



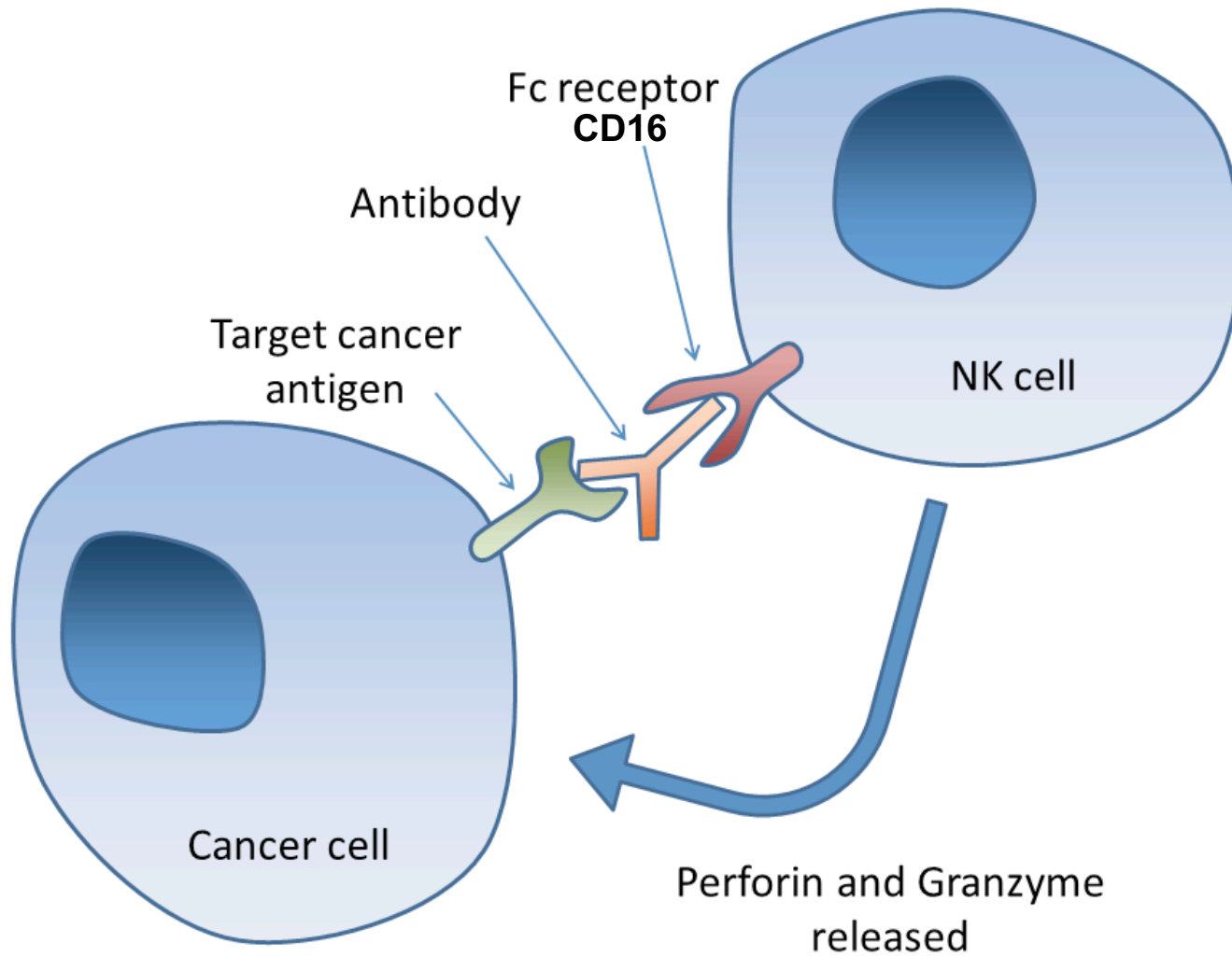
AR Steinman L. 2014.
Annu. Rev. Immunol. 32:257–81

Effetti indiretti:

a Immune-mediated effects of tumour-specific IgG: **FcR-dependent functions**

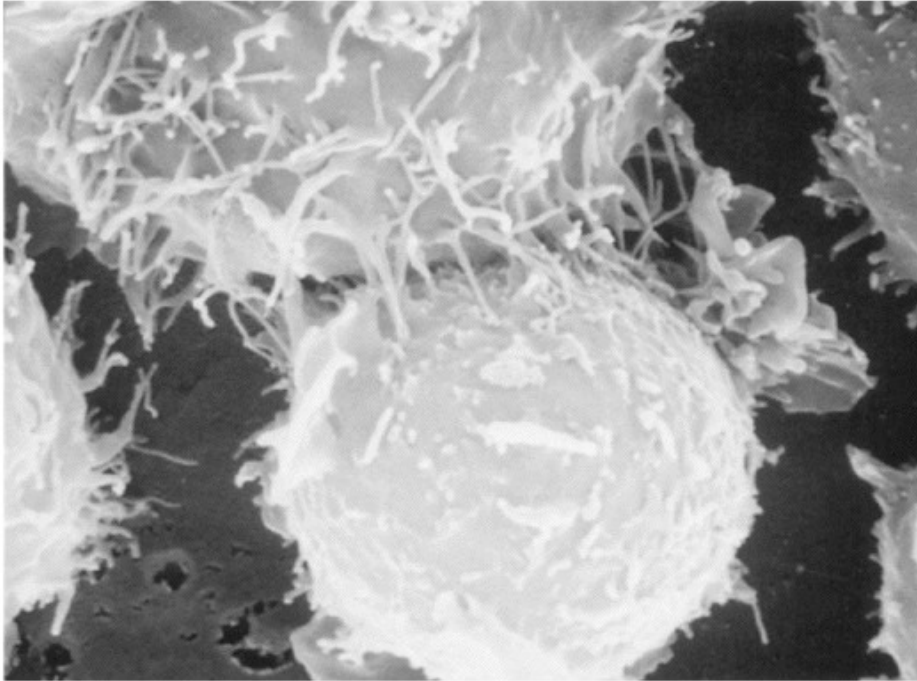


ADCC: antibody-dependent cellular cytotoxicity

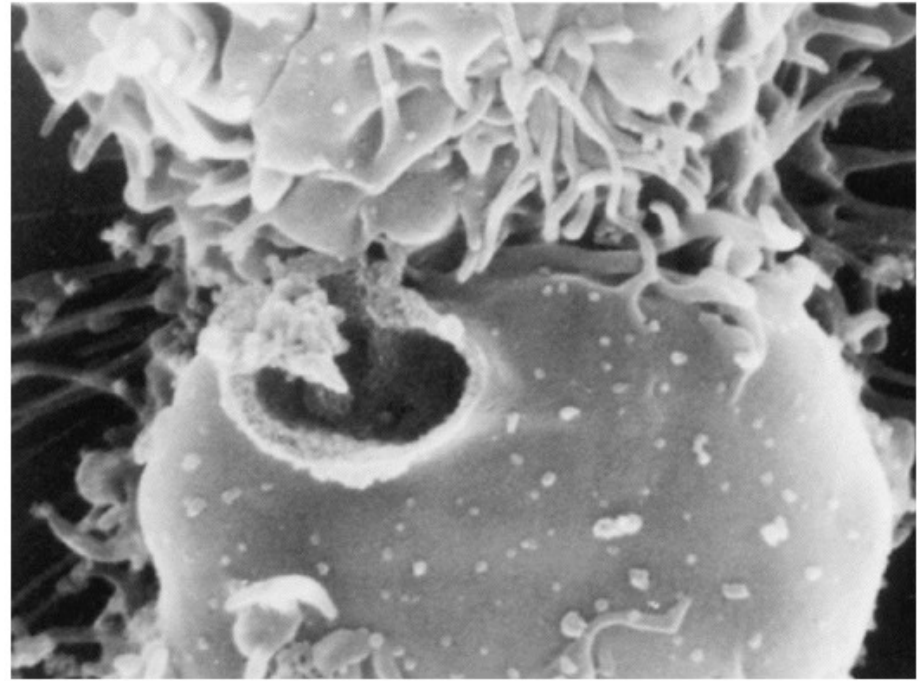


Target cell death: IL BACIO DELLA MORTE

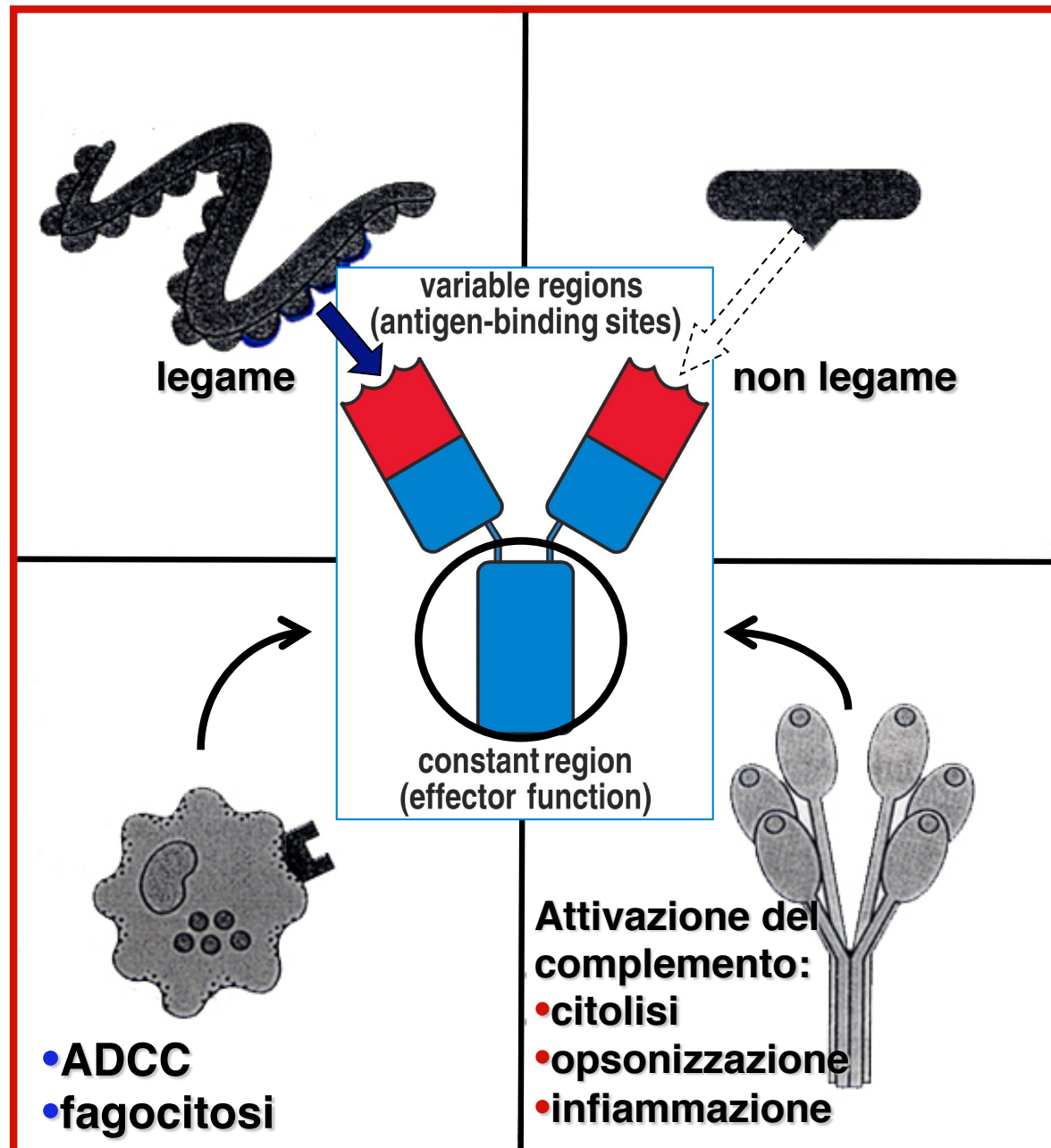
(a)



(b)



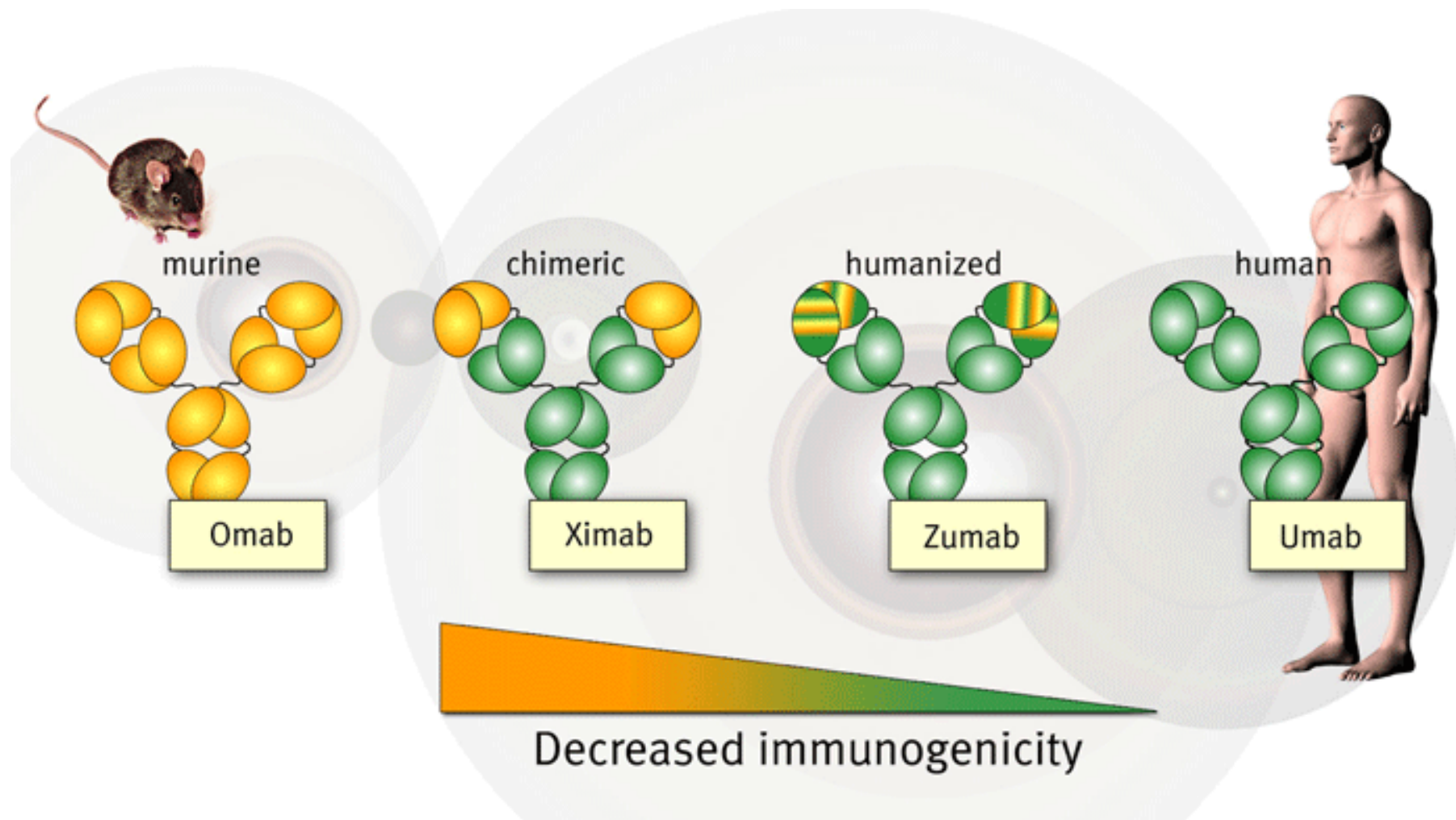
La regione costante dell'anticorpo monoclonale influenza l'emivita e l'innescò di funzioni mediate da FcRs



PROBLEMI RELATIVI ALL' IMPIEGO DI mAb MURINI *in vivo*

- **HAMA human anti-mouse antibodies**
- **mAb murini non attivano efficientemente alcune funzioni effettrici mediate dal frammento Fc (ADCC, complemento)**
- **Ab umani hanno emivita più lunga (legame a FcRn - Brambell receptor-su endotelio, recycling continuo delle Ig)**

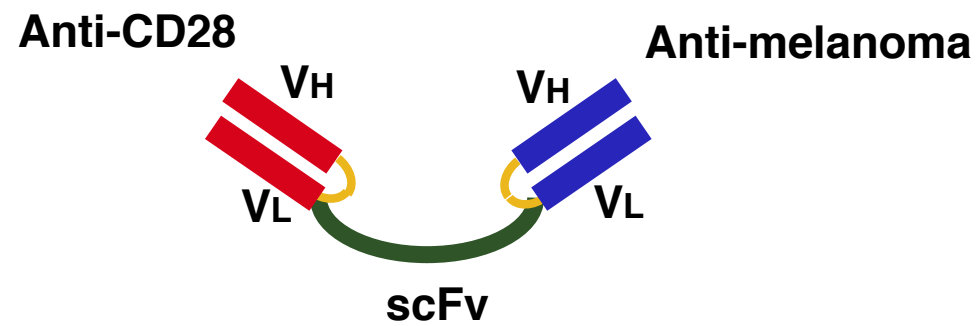
EVOLUZIONE DEGLI ANTICORPI MONOCLONALI TERAPEUTICI



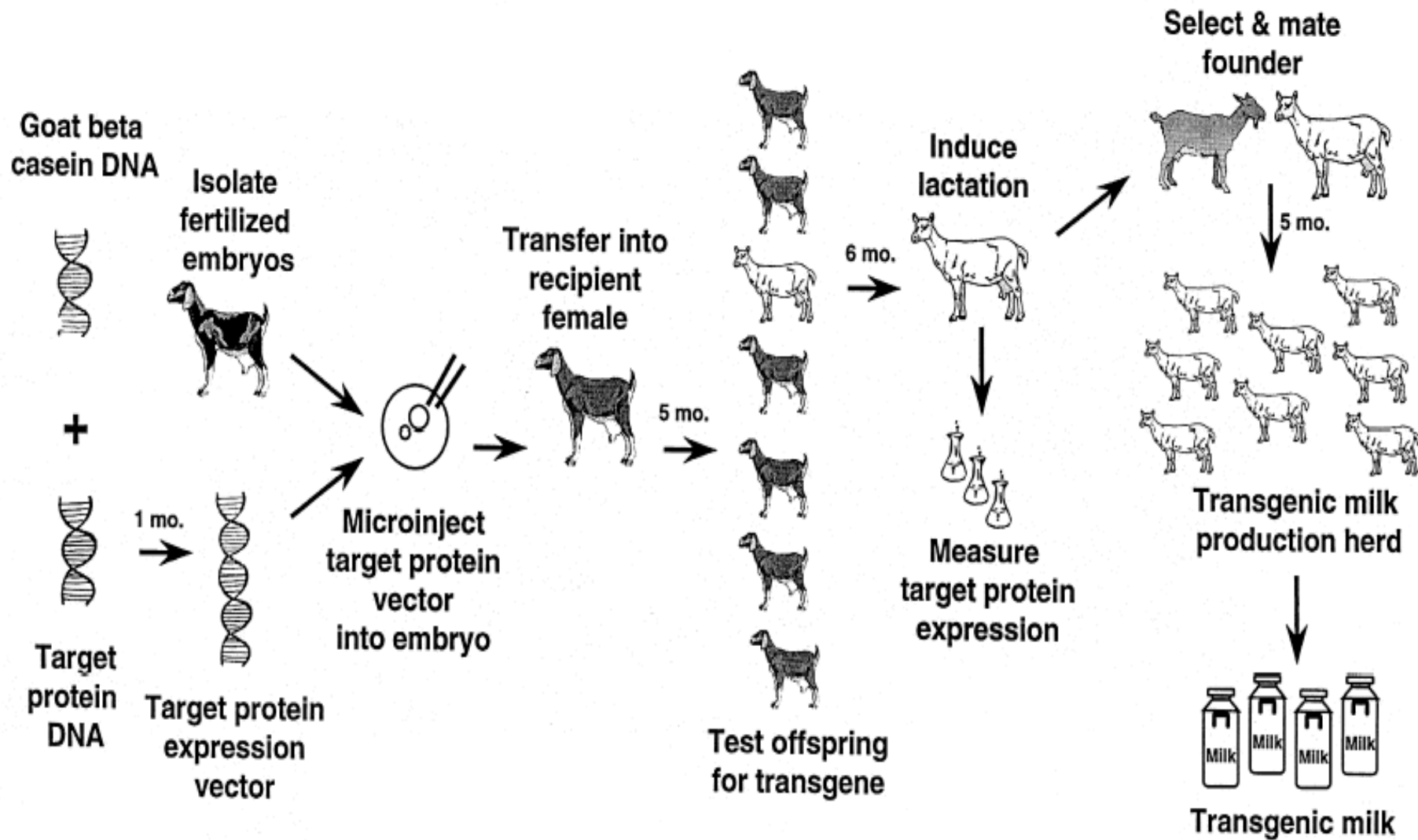
Anticorpi monoclonali modificati (ingegnerizzati):

- TRASFEZIONE LINEE CELLULARI DI MAMMIFERO (COS, CHO...)**
- TRASFEZIONE LINEE CELLULARI DI INSETTO**
- TRASFEZIONE CELLULE VEGETALI (TABACCO TRANSGENICO)**
- LATTE DA ANIMALI TRANSGENICI**

MUCCHE TRASGENICHE PRODUTTRICI DI ANTICORPI BISPECIFICI NEL LATTE

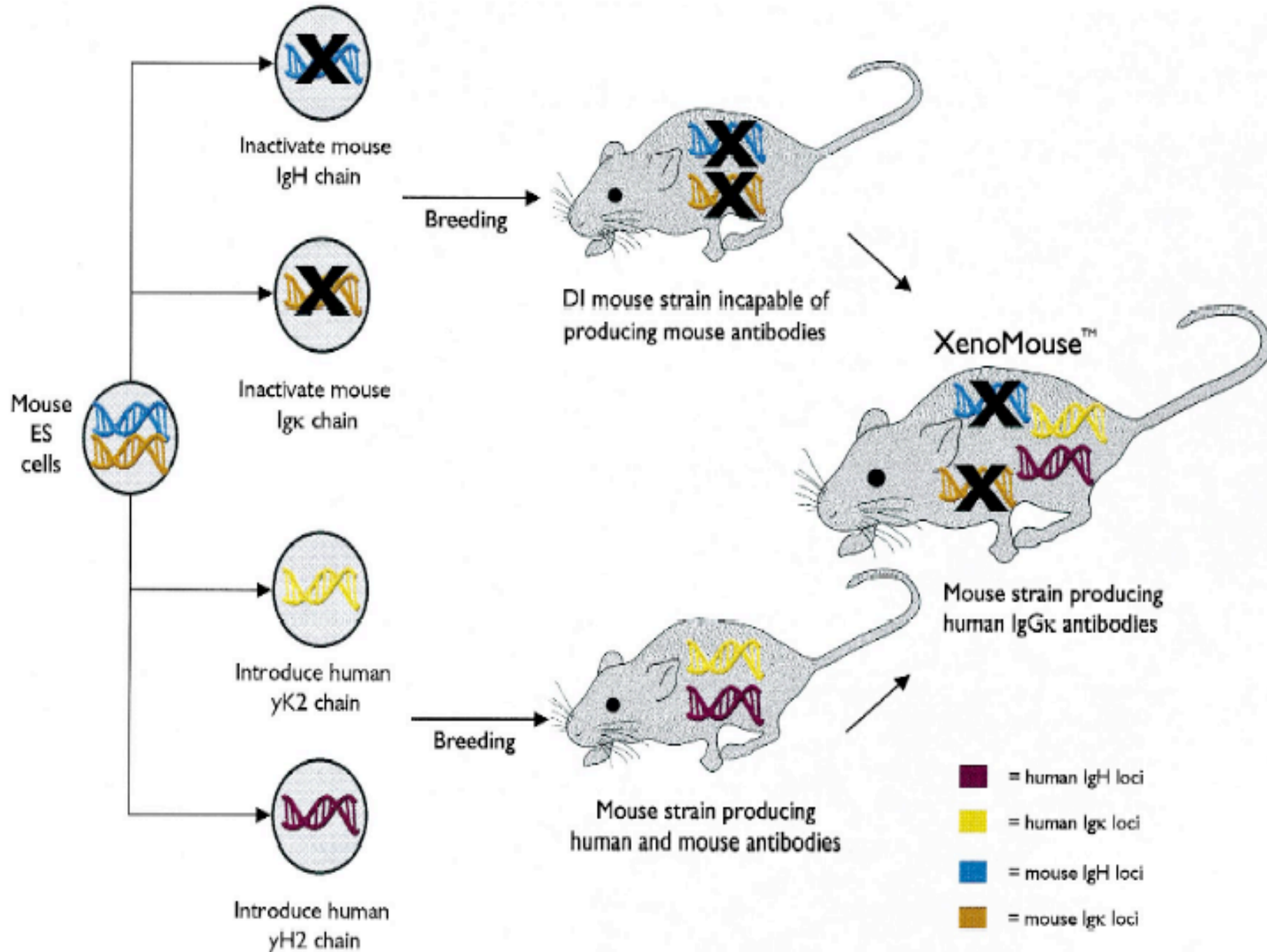


PRODUZIONE DI ANTICORPI NEL LATTE DI CAPRE TRANSGENICHE

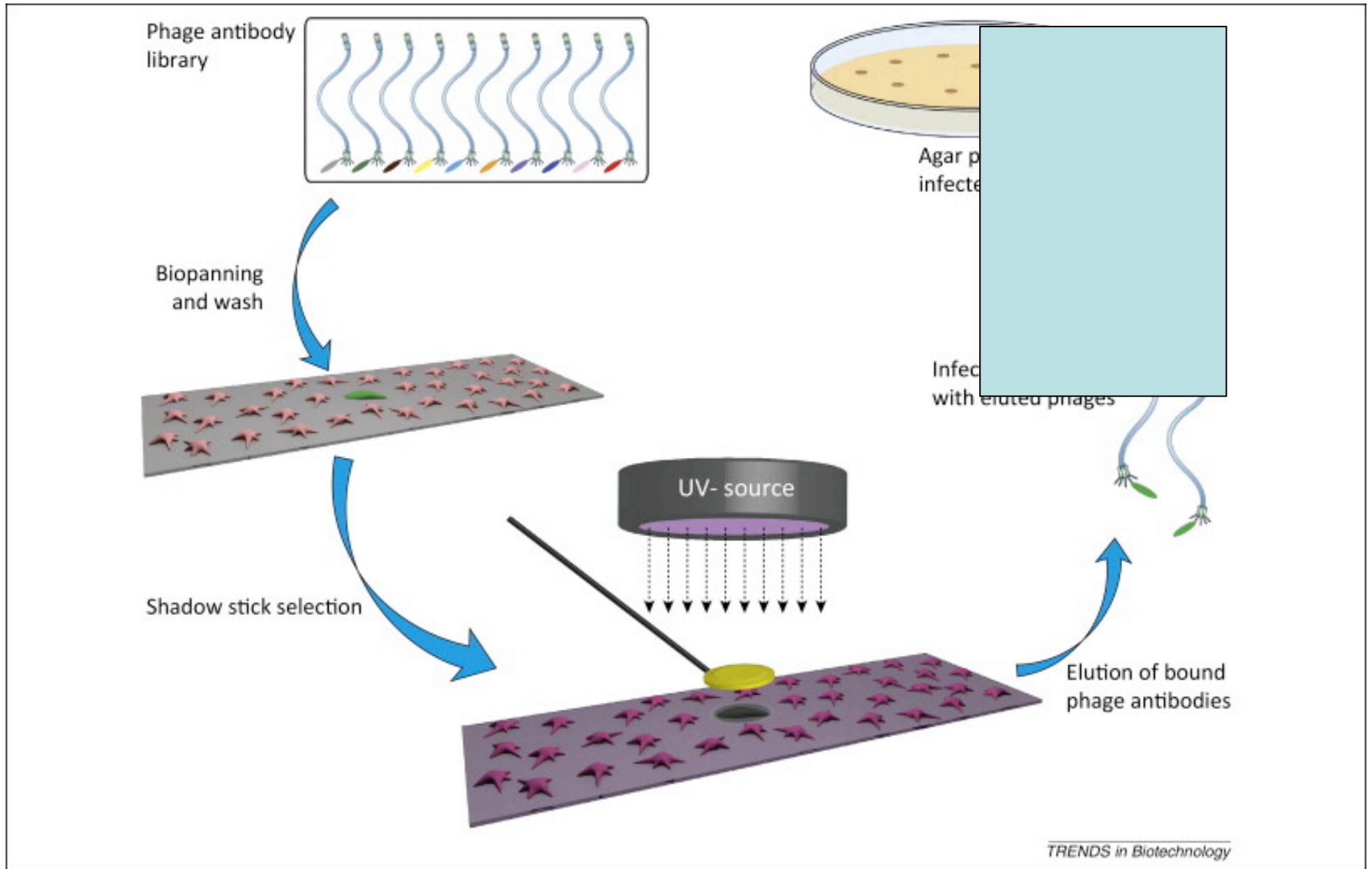


600-800 lt di latte in 300 giorni di lattazione: 5mg/ml !!!!

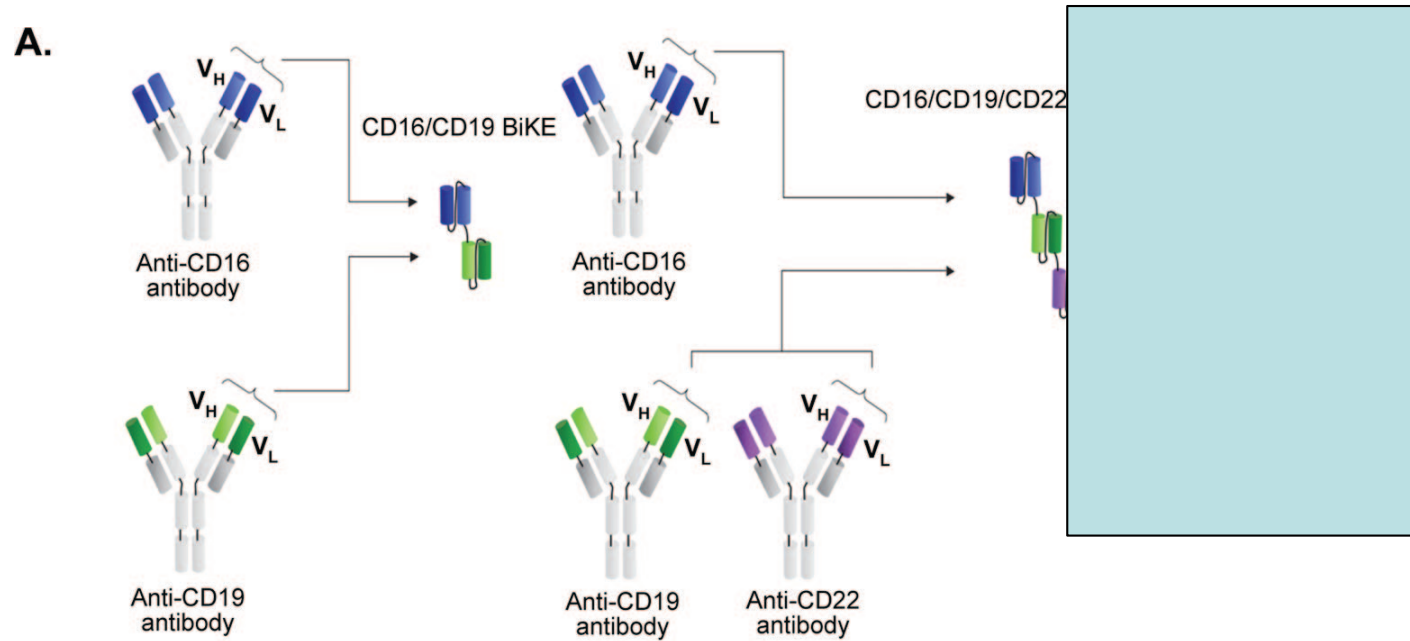
XENOMOUSE: monoclonali umani



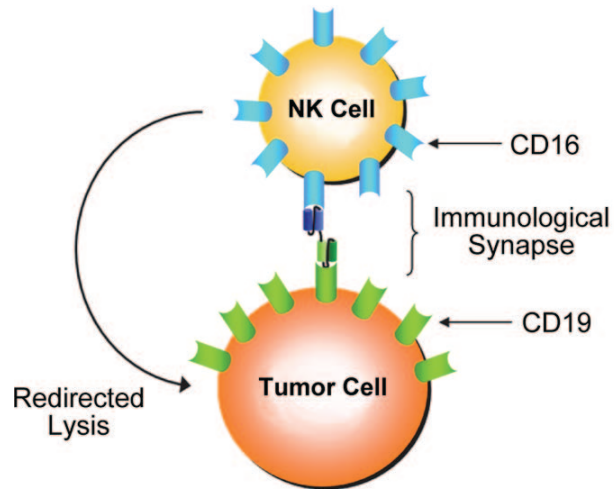
Tecnología del Phage display



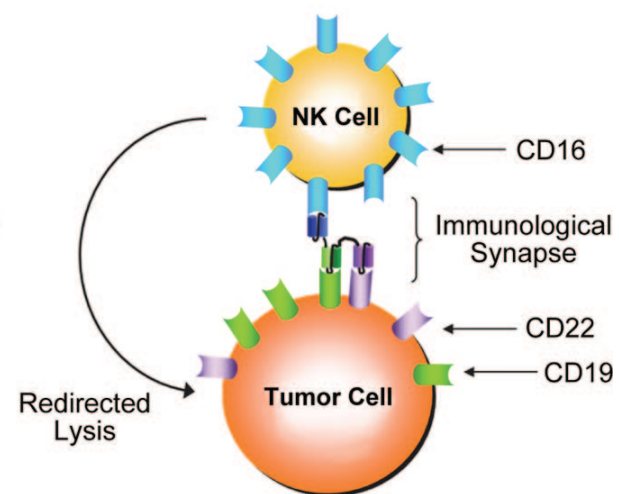
ANTICORPI BIKE and TriKE

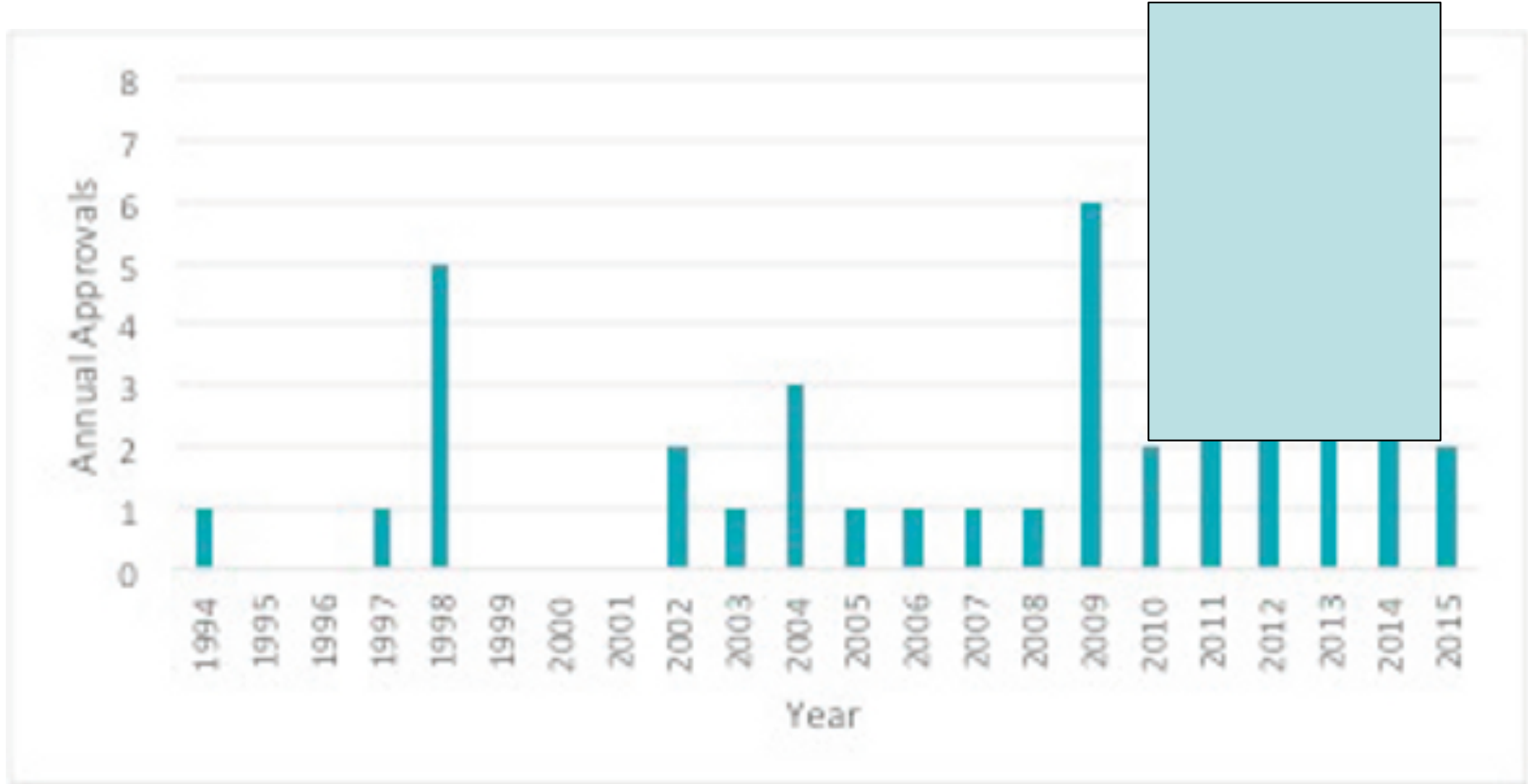


B. BiKE-Mediated Killing



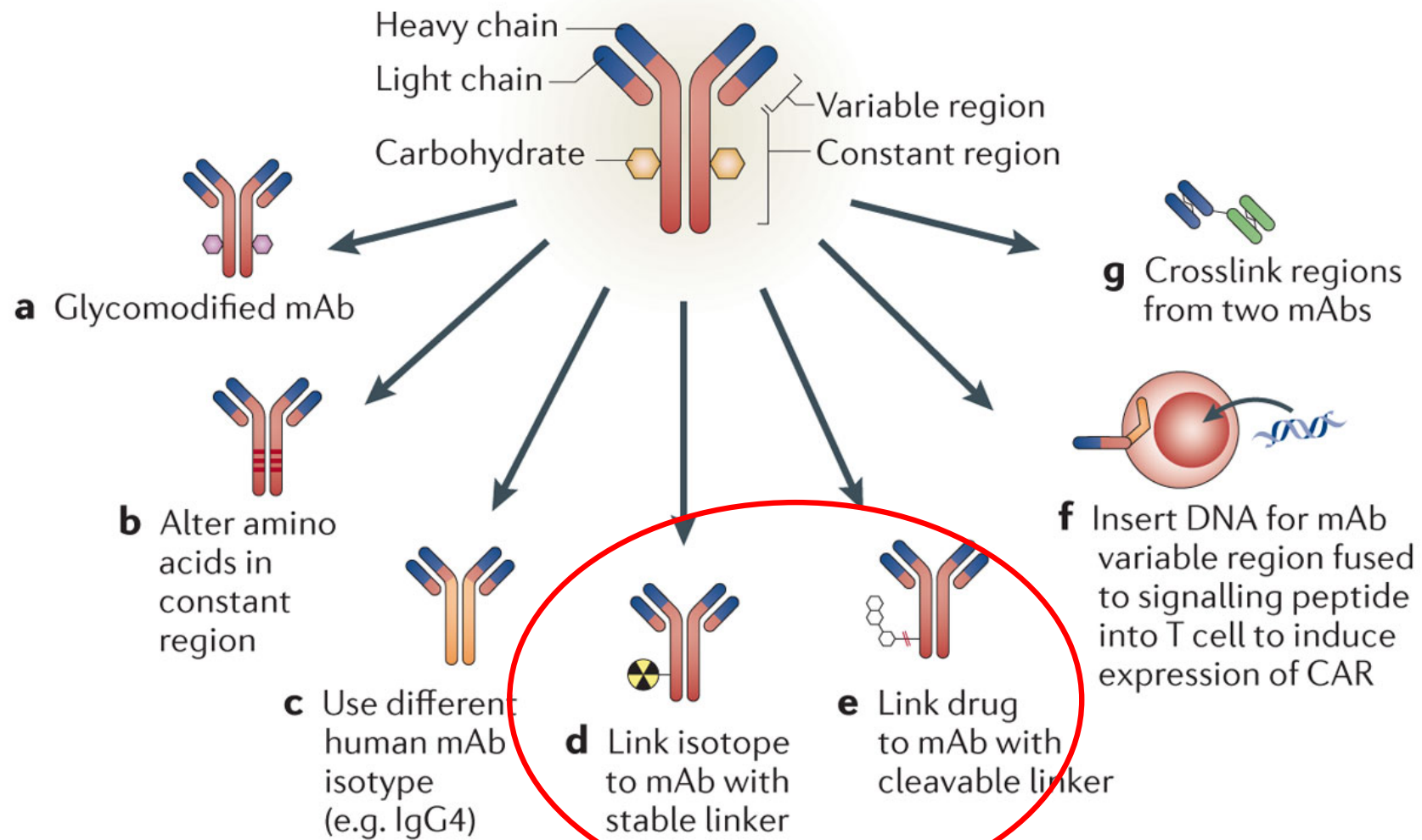
TriKE-Mediated Killing





***MONOCLONAL ANTIBODY-BASED NEW STRATEGIES
IN TUMOR THERAPY***

Modifying monoclonal antibody structure



Structure of an antibody-drug conjugate (ADC)

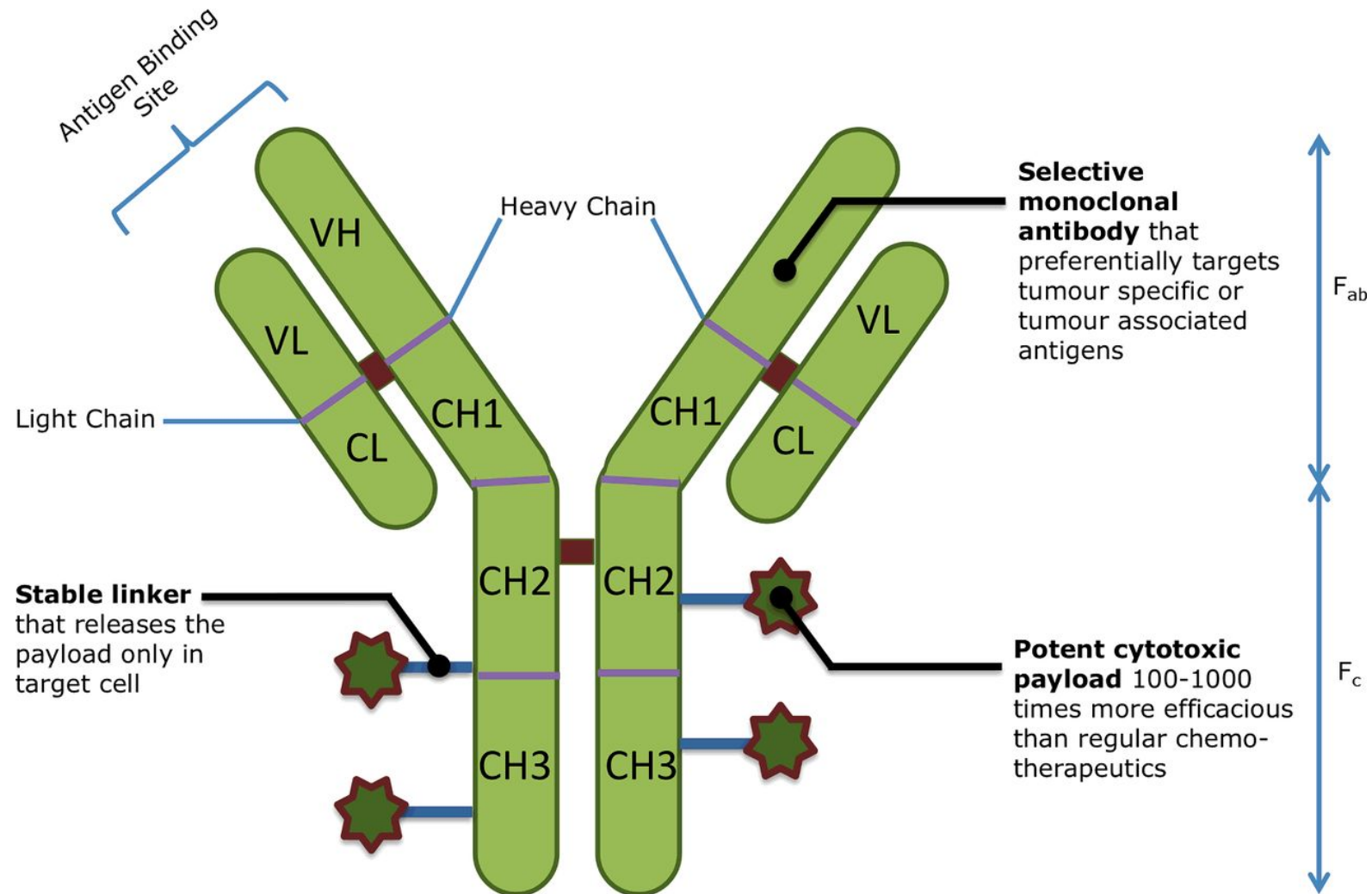


Table 1. Cytotoxic Drug Classes Commonly Used in Antibody-Drug Conjugates

Drug class	Mechanism of action	Drug examples/ Conjugate name
Auristatin	Microtubule disruption: binds to and inhibits polymerization of tubulin	Monomethyl auristatin E (MMAE)/ Vedotin Monomethyl auristatin F (MMAF)
Calicheamicin	DNA disruption: binds to minor groove and cleaves DNA	Calicheamicin γ 1/Ozogamicin
Maytansine	Microtubule disruption: binds to and inhibits polymerization of tubulin	DM1 (mertansine)/ Emtansine DM4

MMAE = monomethyl auristatin E; MMAF = monomethyl auristatin F.

Mechanism of action of antibody-drug conjugates

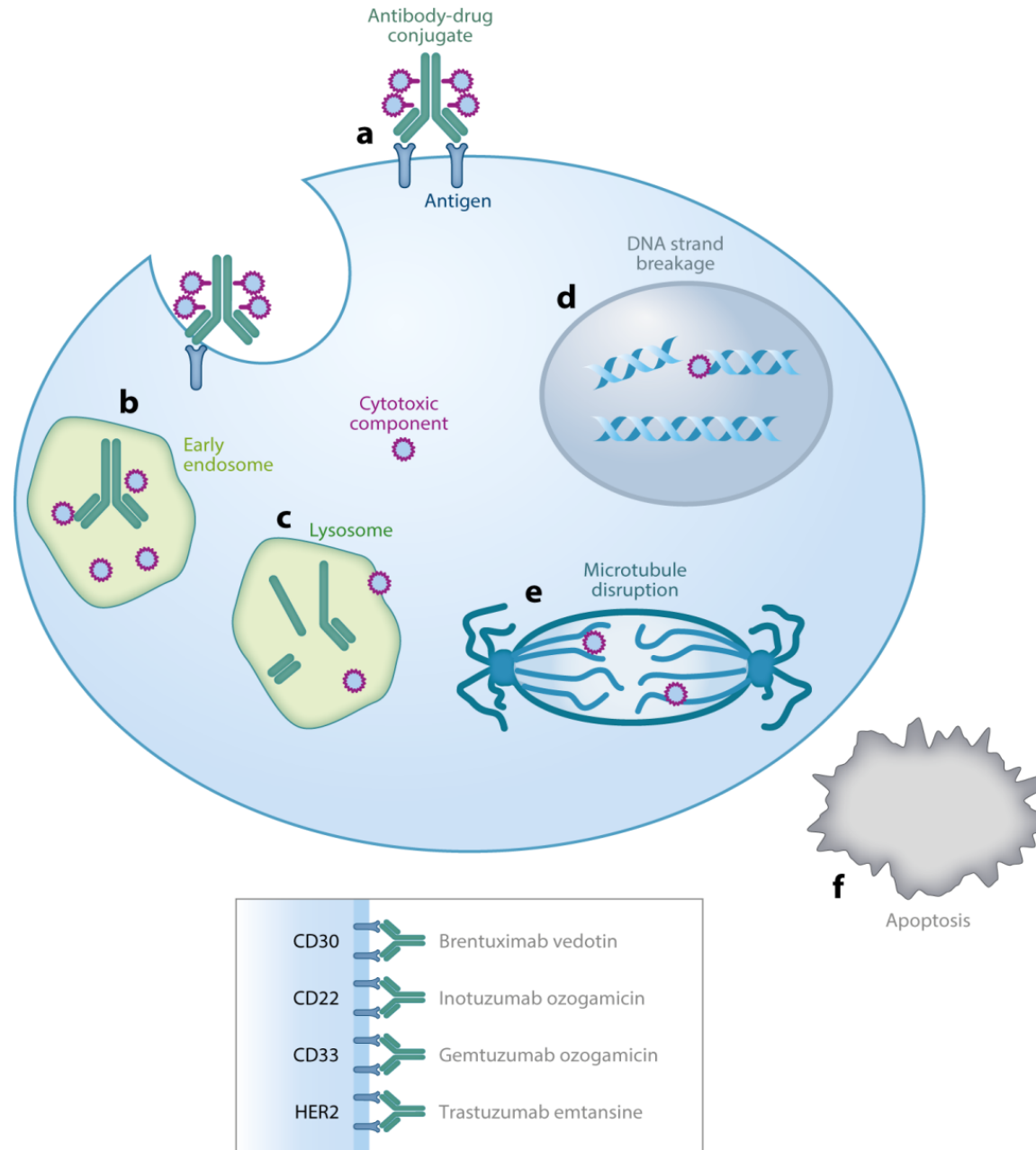
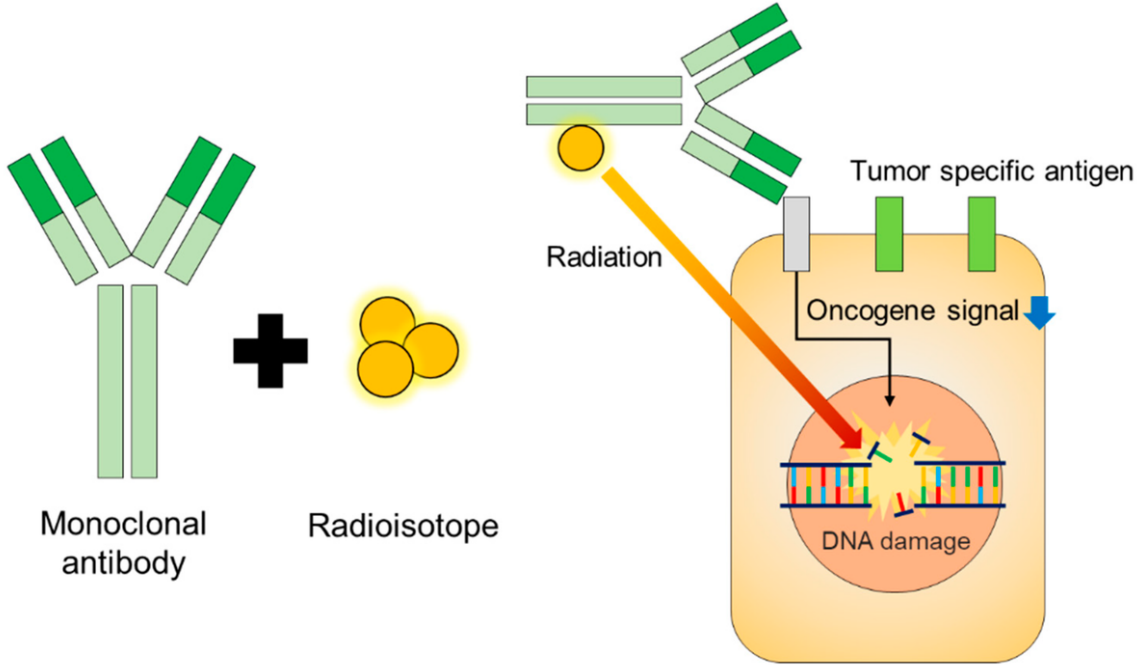


Table 4. ADCs Approved or in Clinical Trials

Drug Name (Alternate Names)	Sponsor	Antibody	Linker	Payload	Target Antigen	Development Stage	Indication
Adcetris (brentuximab vedotin, SGN-35)	Seattle Genetics	Chimeric IgG1	VC	MMAE	CD30	Approved	HL, sALCL
Kadcyla (ado-trastuzumab emtansine, T-DM1)	Genentech	Humanized IgG1	SMCC	DM1	HER2 (ErbB2)	Approved	HER2-positive breast cancer

Radioimmunotherapy (RIT)



Radioimmunotherapy (RIT)

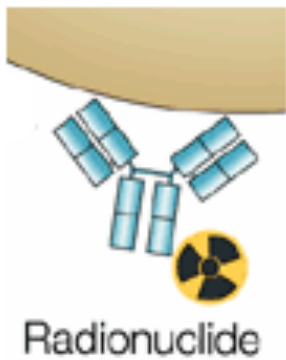


Table 1. Principal Radionuclides Used for Antibody-Targeted Imaging and Therapy

Radionuclide	T _{1/2} (h)	Emissions	E _{max} (keV)	Maximum Range in Soft Tissue (mm)
Fluorine 18	1.83	β ⁺	633	3.1
Gallium 68	1.13	β ⁺	1,899	9.8
Copper 64	12.7	β ⁺	653	3.2
		β ⁻	579	2.8
Zirconium 89	78	β ⁺	902	4.6
Iodine 124	100	β ⁺	1,535 and 2,138	7.9 and 10.9
Scandium 44	3.97	β ⁺	1,473	7.6
Iodine 131	193	β ⁻	610	2.9
		γ	362	
Yttrium 90	64	β ⁻	2,250	11
Lutetium 177	162	β ⁻	498	2.0
		γ	208	
Copper 67	62	β ⁻	392–577	1.8
		γ	184	
Bismuth 213	0.76	→ α	8,400	0.1
		γ	440	
Astatine 211	7.2	→ α	5,870 and 7,450	0.055–0.080
		X	77-92	

GLI ANTICORPI MONOCLONALI CONSENTONO UNA RADIOTERAPIA SELETTIVA

Table 2 | **Radioimmunotherapeutics in the clinic**

Therapy (trade name)	Indication	Ligand	References
⁹⁰ Yttrium-ibritumomab tiuxetan (Zevalin)	NHL (approved)	Mouse anti-CD20 IgG ₁	9,111
⁹⁰ Yttrium-ibritumomab tiuxetan versus rituximab (Zevalin versus Rituxan)	Relapsed or refractory low-grade follicular NHL (Phase III)	Mouse and human/mouse chimeric anti-CD20 IgG ₁	10,112
¹³¹ Iodine-tositumomab (Bexxar)	NHL (Phase III)	Mouse anti-CD20 IgG _{2a}	29,30,31
⁹⁰ Yttrium-epratuzumab (hLL2)	NHL and B-cell lymphoma (Phase I/II)	Humanized anti-CD22	31,33
¹³¹ Iodine-Lym1 (Oncolym)	Diffuse large B-cell lymphoma (Phase I)	Mouse anti-HLA-DR10 β-subunit	30,32
²¹³ Bismuth-HuM195	Acute myeloid leukaemia (Phase I/II)	Humanized anti-CD33	35,37
⁹⁰ Yttrium-daclizumab	T-cell leukaemia (Phase I/II)	Humanized anti-Tac/CD25	National Cancer Institute trials NCI-96-C-01471, NCI-97-C-0110F

NHL, non-Hodgkin's lymphoma.

α-particle emitters in solid tumours

α-immunotherapy

Therapy antibody	Antigen target	Study population	Special feature	Main findings	Refs
²¹³ Bi-9.2.27	Glial antigen 2 (NG2)	Stage IV or in transit melanoma	Long-term evaluation of response?	10% PR, 8% SD, no MTD	133
²¹³ Bi-9.2.27	Glial antigen 2 (NG2)	Stage IV or in transit melanoma	First-in-human direct injection	Antitumour effect at 600 μCi. Safe, no MTD, activity administered 150 to 1350 mCi	134
²¹¹ At-ch81C6	Tenascin	Primary brain tumours	18 patients 71–347 MBq post-resection, delivery into surgically created resection cavity	No MTD achieved, no DLT. No haematological >grade 2. Limited neurotoxicity. Determined biodistribution. Median survival 54 weeks for GBM and 52 weeks for AA and 116 weeks for OD, 2 of 14 GBM survived ~3 years. Proof-of-concept regional targeted radiotherapy with ²¹¹ At	81
²¹³ Bi 9.2.27	Glial antigen 2 (NG2)	22 patients with stage IV melanoma	Phase I dose escalation. 1.5 to 25.6 mCi	Well tolerated; no DLT. 14% PR, 50% SD	135

AA, anaplastic astrocytoma; DLT, dose-limiting toxicity; GBM, glioblastoma; MTD, maximum tolerated dose; OD, oligodendroglioma; PR, partial remission; SD, standard deviation.

Intrathecal RIT imaged quantitatively with PET imaging

