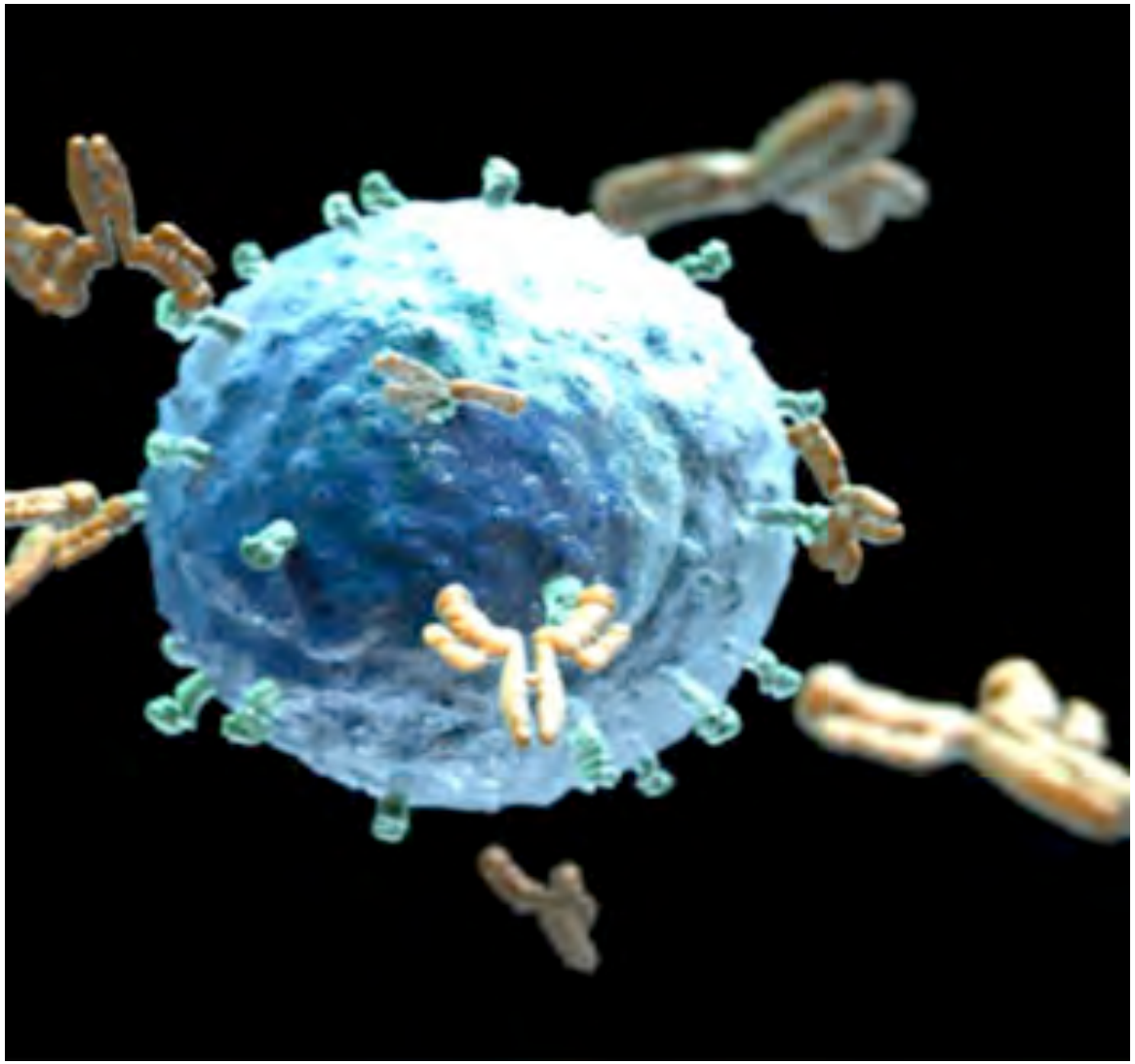
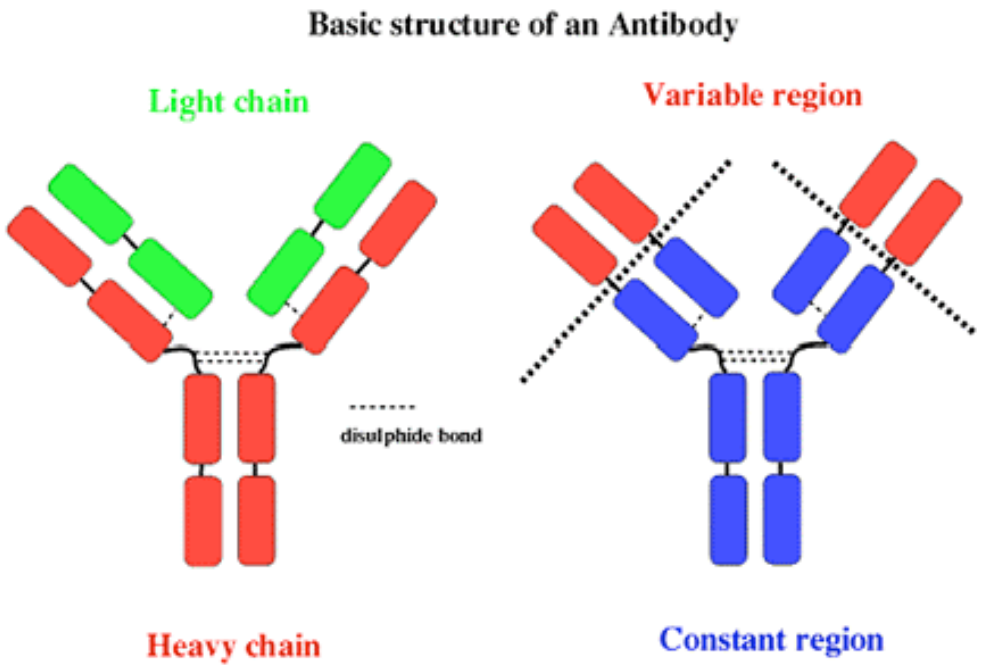
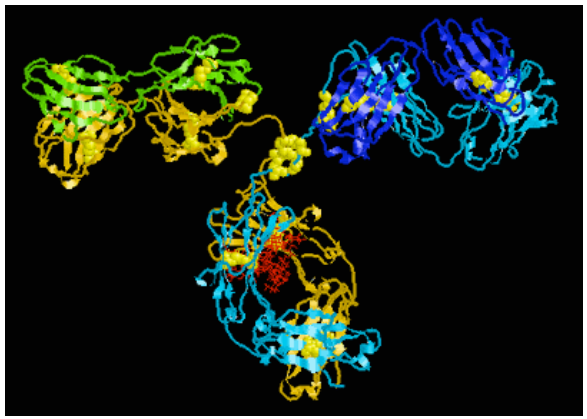
Monoclonal antibodies (mAbs)



mAbs = insieme di anticorpi identici fra loro in quanto prodotti da un solo tipo di cellule B (cloni cellulari)





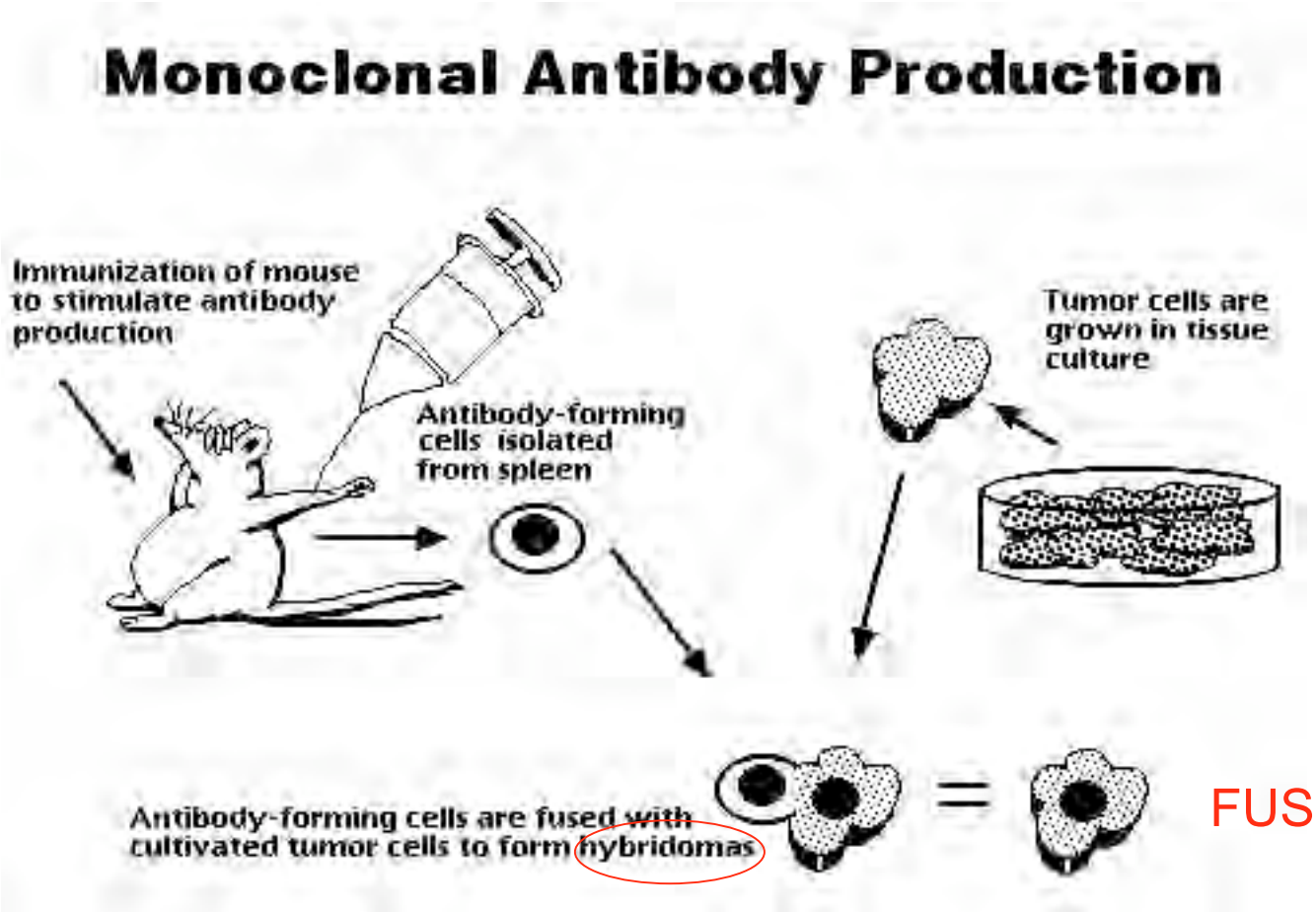
Use of monoclonal antibodies (mAbs)

- **Diagnostics**: quantitation of antigens relevant to diseases (hormones, microbial antigens, toxins, etc…), quantitation of cell subsets, identification of pathological tissues, cells, etc…
- **Therapeutics**: targeting of drugs to cells to be killed (tumors, virus-infected cells, autoreactive lymphocytes, etc…), targeting of receptors to be blocked or stimulated
- **Laboratory reagents**: immuno-isolation, -fractionation, -precipitation, -identification, -purification etc… of cells, organelles or any cellular component or molecule
- **Other**

Georges Köhler
César Milstein
Nature, 1975

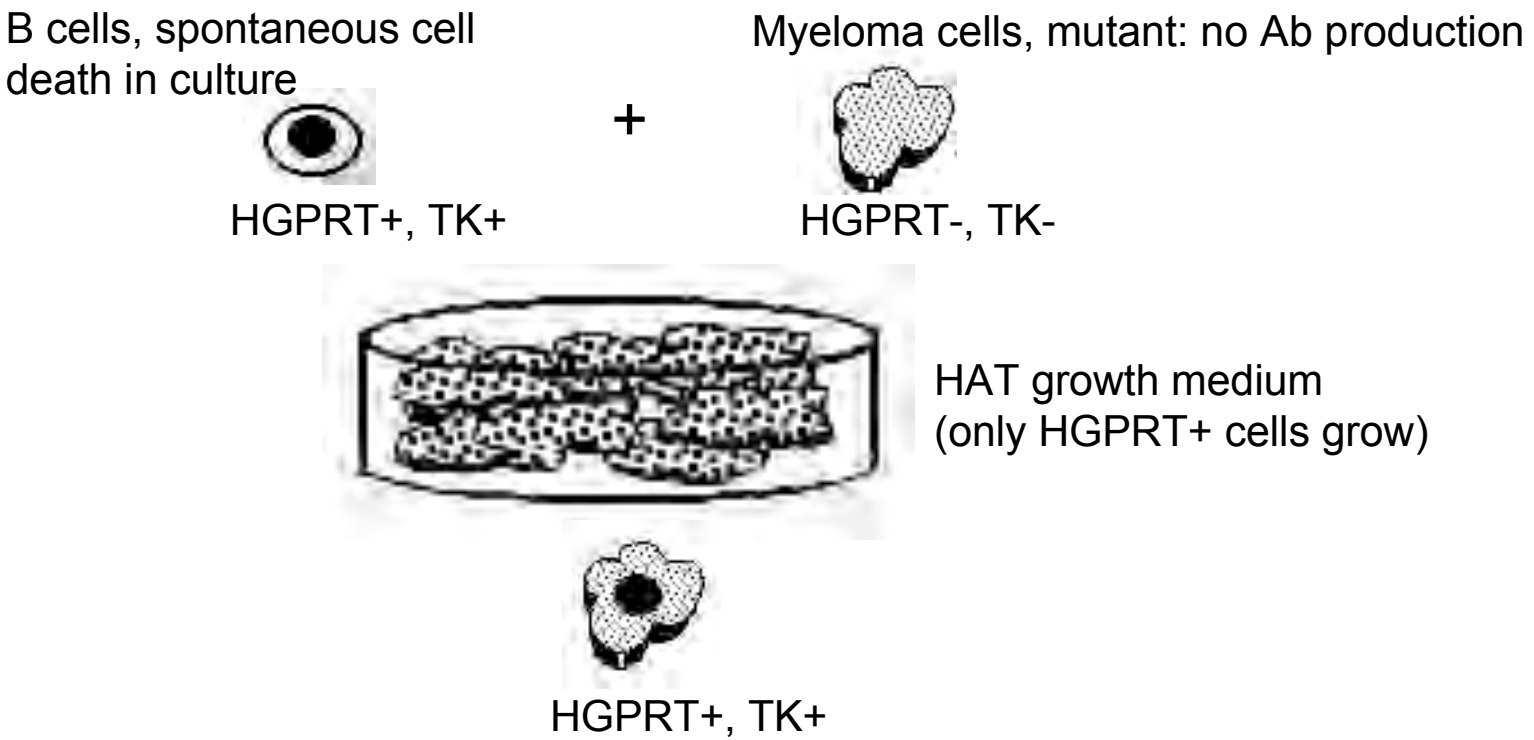
Premio Nobel 1984
"per le loro teorie sulla specificità nello sviluppo e nel controllo del sistema immunitario e la scoperta del principio per la produzione di anticorpi monoclonali”

Monoclonal Antibody Production



FUSION

Monoclonal Antibody Production



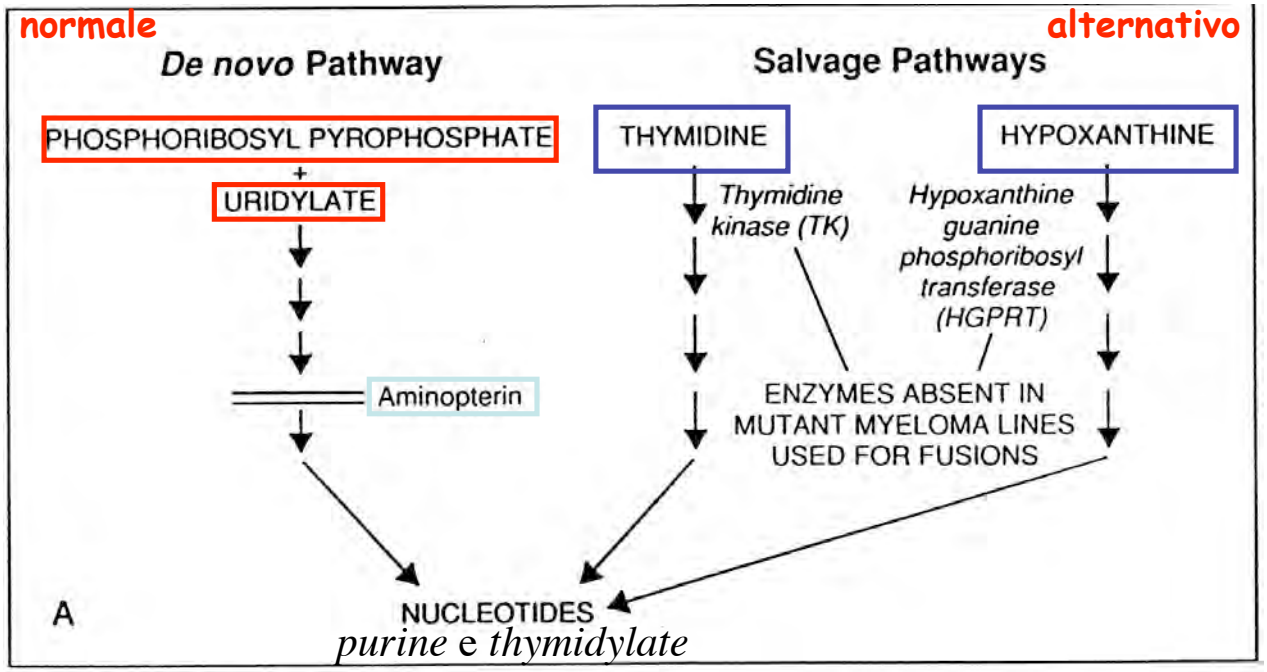
HGPRT: Hypoxanthine Guanine PhosphoRibosyl Transferase
TK: Thymidine Kinase
HAT: Hypoxanthine, Aminopterin, Thymidine

Le cellule di mieloma ($TK^-/HGPRT^-$) non secernono Ac e muoiono in HAT medium, perché mancano del gene funzionale (presente nelle cellule normali) richiesto per la sintesi di DNA nel terreno HAT. Questa linea cellulare è stata appositamente creata con un difetto nel normale pathway della sintesi nucleotidica.

Principi della selezione mediante HAT

Infatti, in condizioni normali, le cellule sintetizzano i nucleotidi *purine* e *thymidylate* (componenti indispensabili per la sintesi del DNA) a partire da *phosphoribosyl pyrophosphate* e *uridylate*; ma il trattamento con farmaci anti-folato quali l'*aminopterin* inibisce tale sintesi secondo il pathway normale, obbligando le cellule stesse ad usare un pathway alternativo.

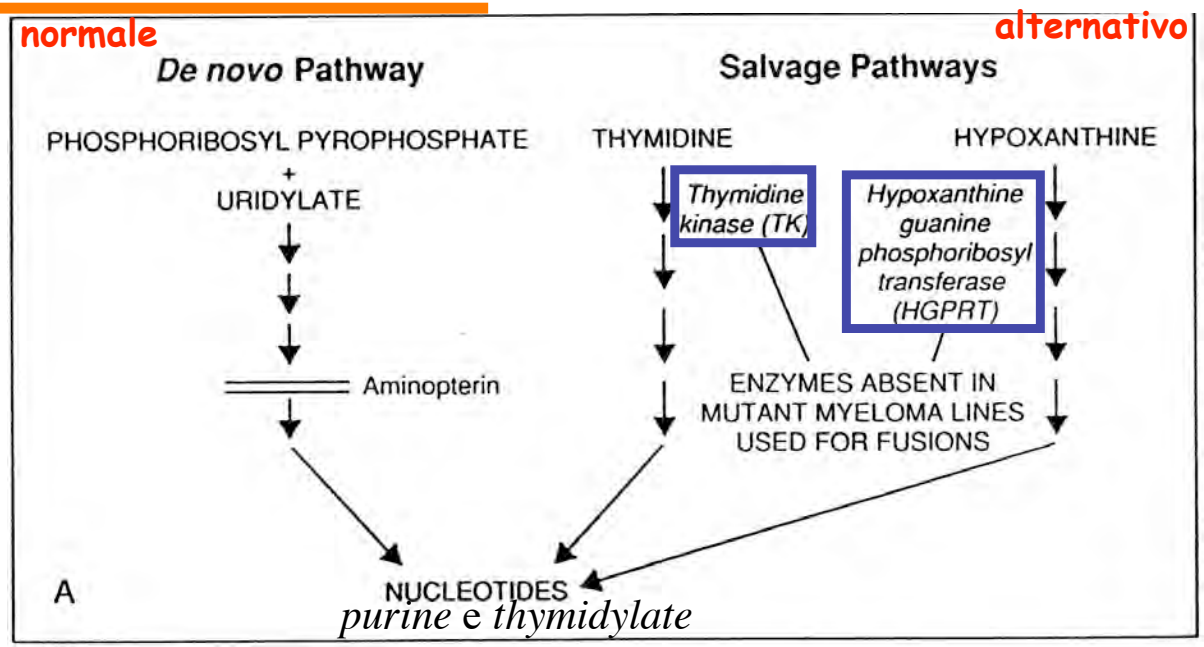
Il pathway alternativo richiede (per la sintesi di *purine* e *thymidylate*) l'apporto esogeno di *hypoxanthine* e *thymidine*, che infatti sono insieme all' *aminopterin* i componenti del medium di selezione denominato HAT.



A. *Pathways of purine synthesis.*

Per sintetizzare –mediante *hypoxanthine* e *thymidine*- i sopracitati nucleotidi, sono indispensabili i rispettivi enzimi *hypoxantine phosphoribosyl guanine transferase* (HGPRT) e *thymidine kinase* (TK) che sono normalmente presenti negli splenociti (e quindi negli ibridomi che ne derivano), ma che mancano appositamente nelle linee di mieloma usate come partner di fusione.

Ne consegue che dopo la fusione solo gli ibridomi riescono a sopravvivere nel medium HAT.

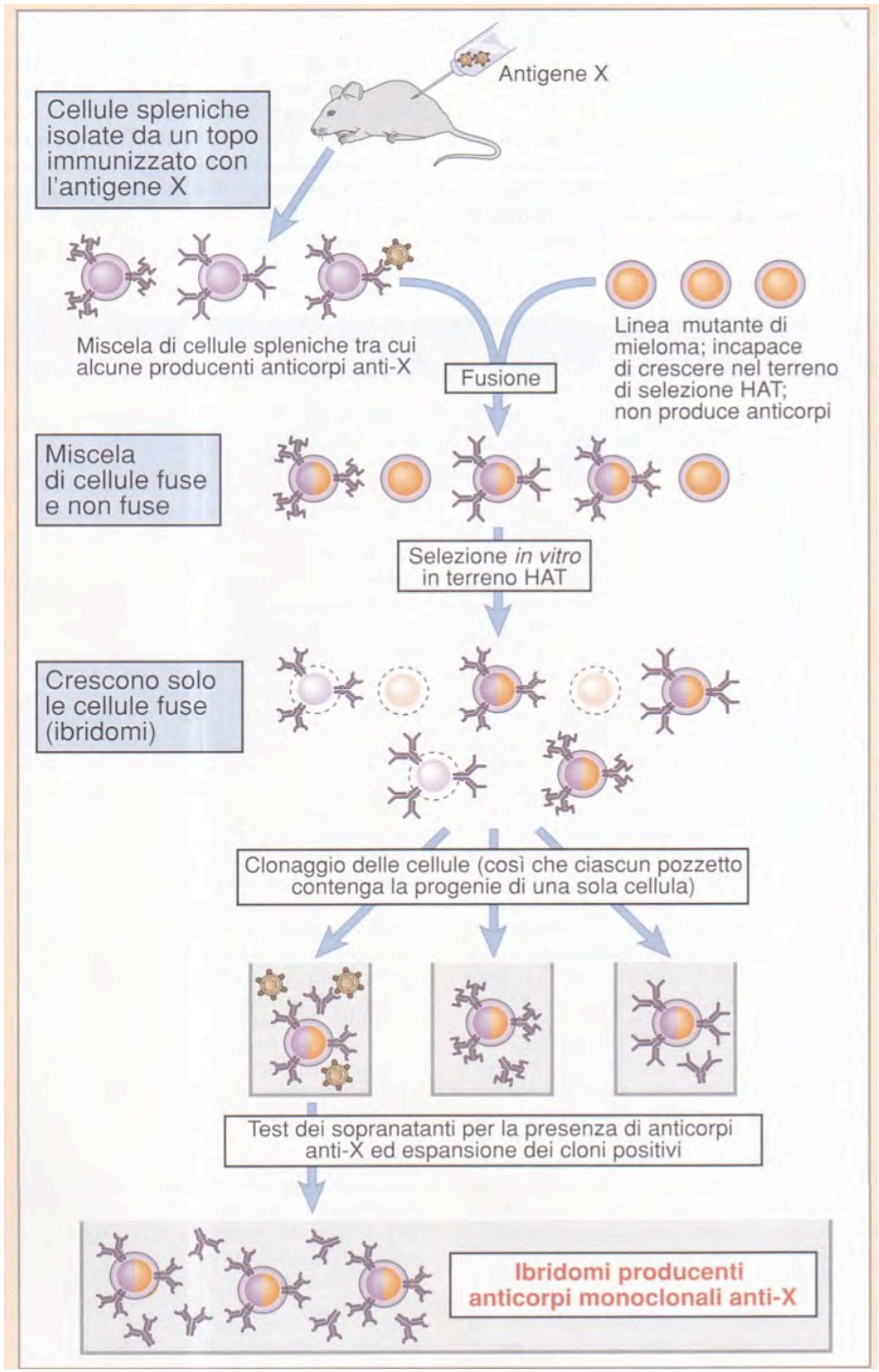


A. *Pathways of purine synthesis.*

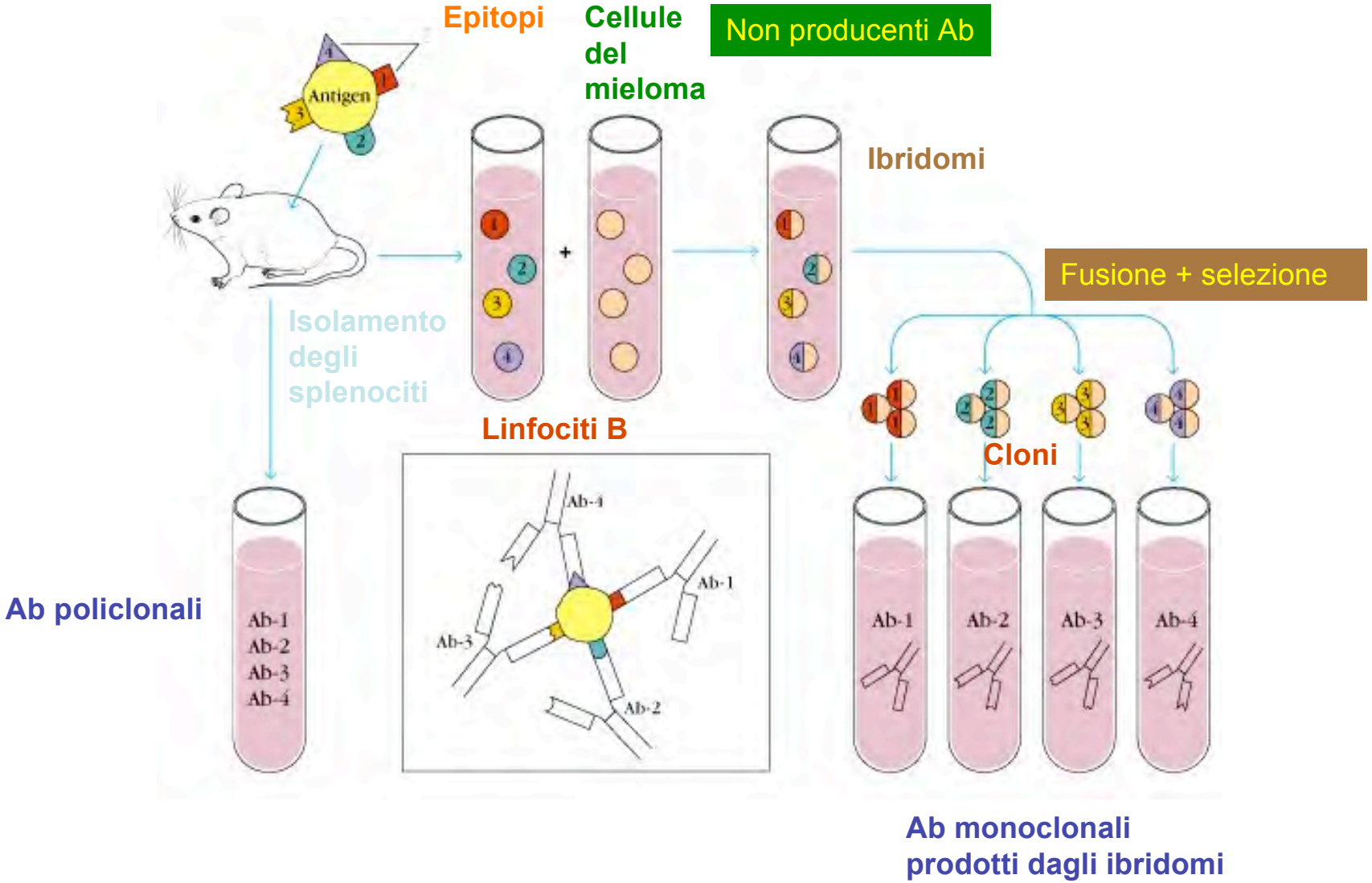
FUSIONE

SELEZIONE

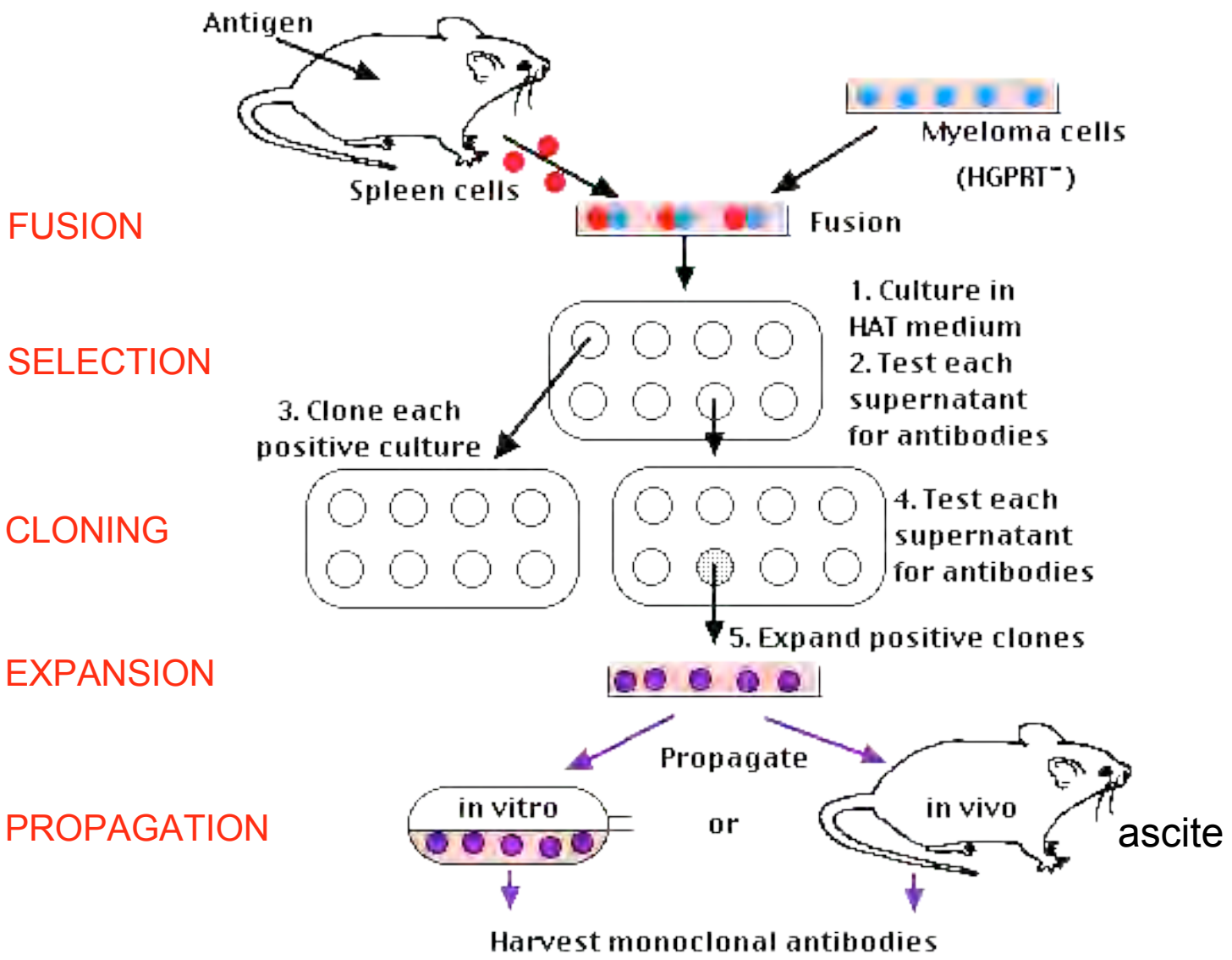
CLONAGGIO



Preparazione degli anticorpi monoclonali

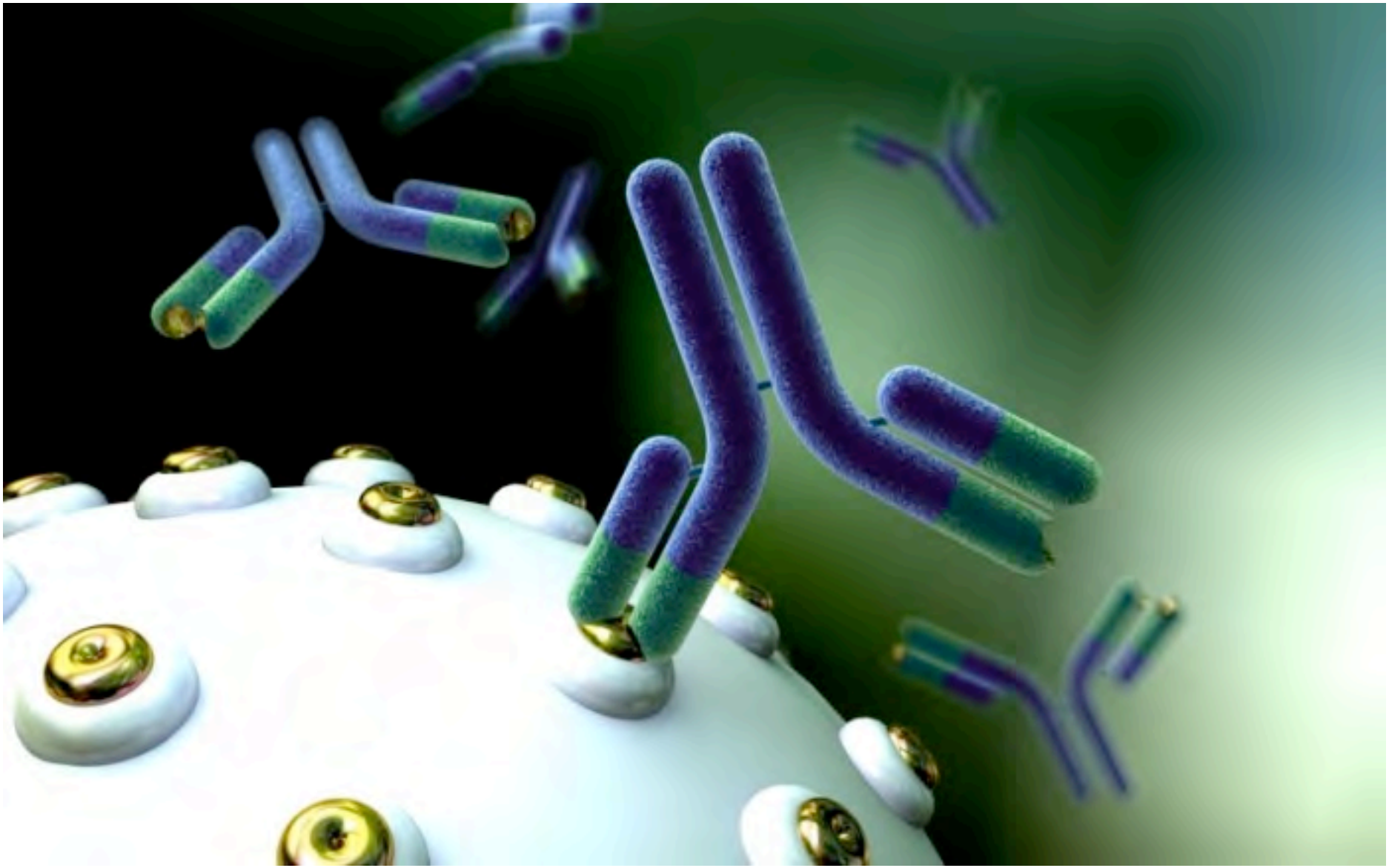


Monoclonal Antibody Production

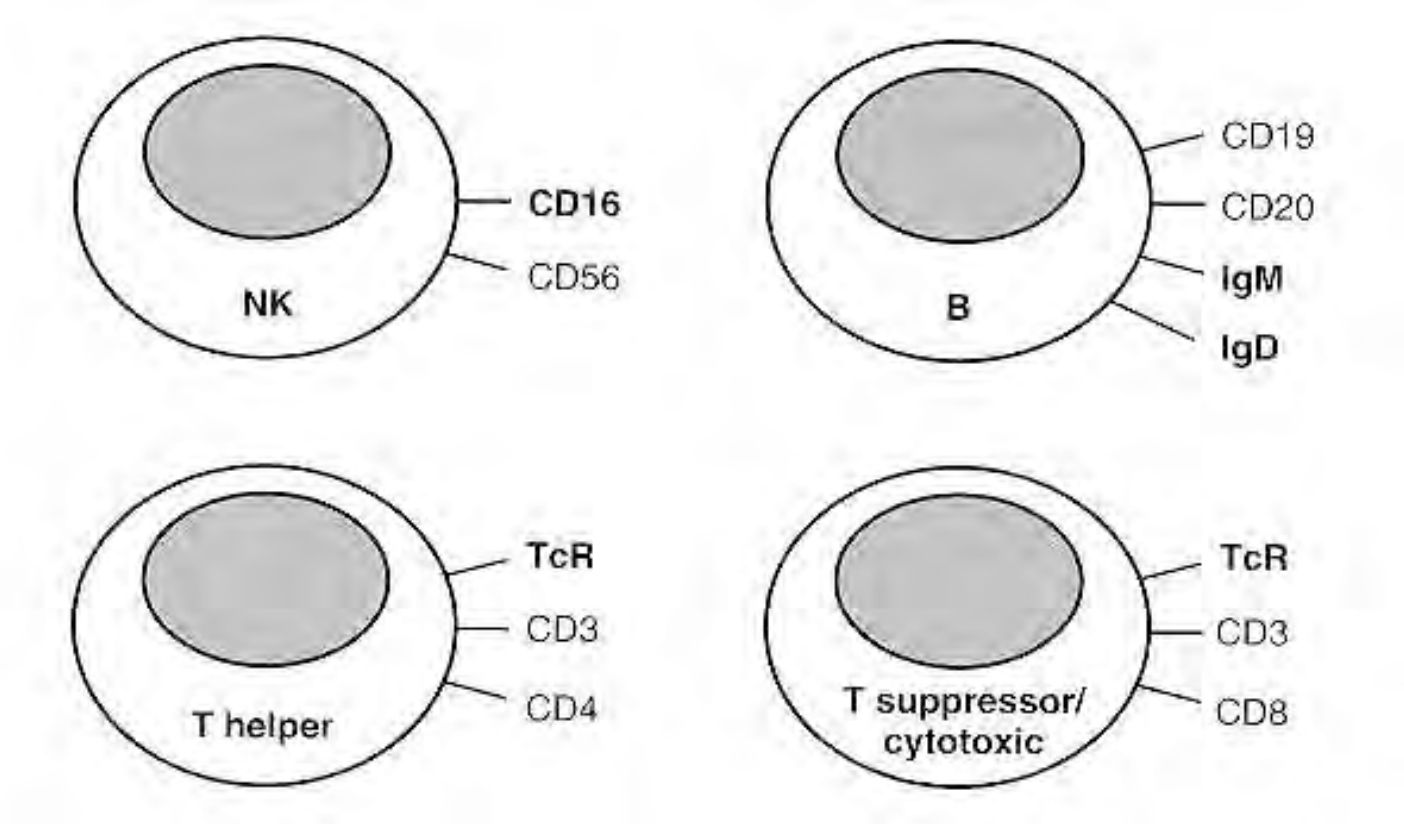


Growth of mAbs-producing cells (hybridomas)

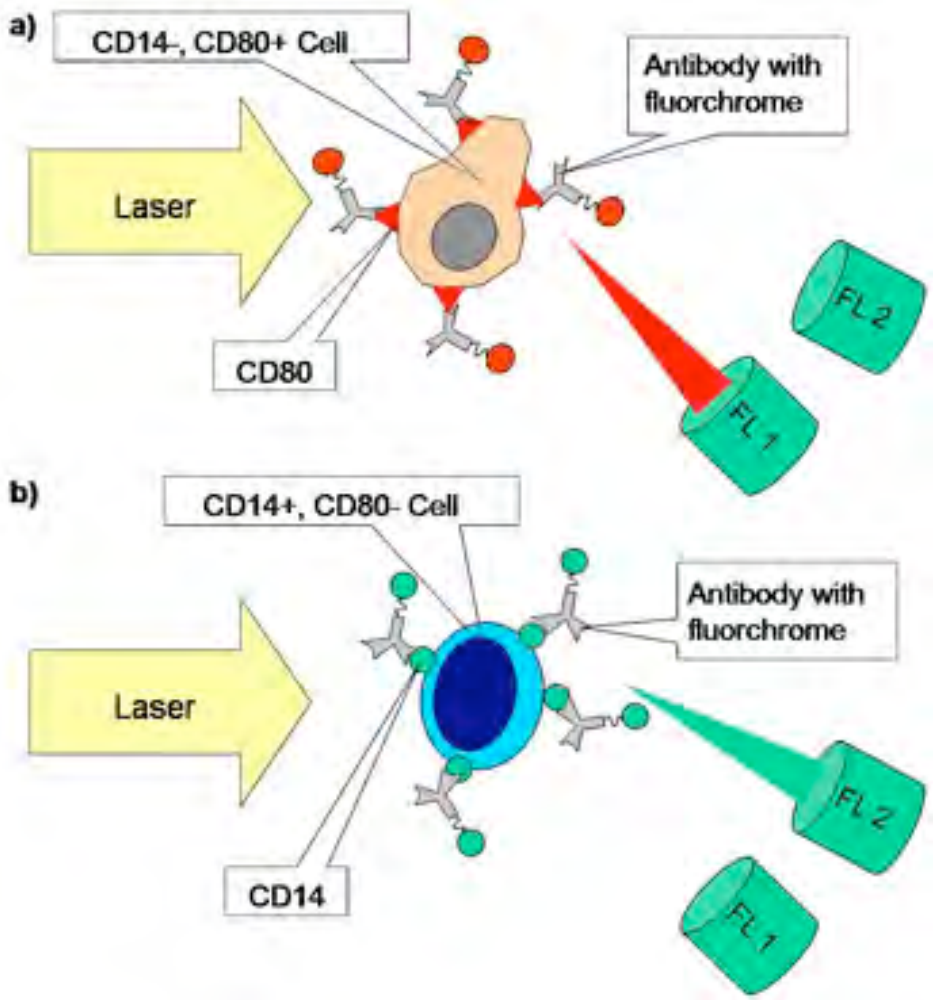
mAbs production



Major lymphocyte subsets



Fluorochrome-conjugated antibodies



Sistemi di screening

1-ELISA (Enzyme-Linked Immunosorbent Assay) diretto o sandwich

2-RIA (Radio Immuno Assay)

3-Immunoperossidasi (Ipx)

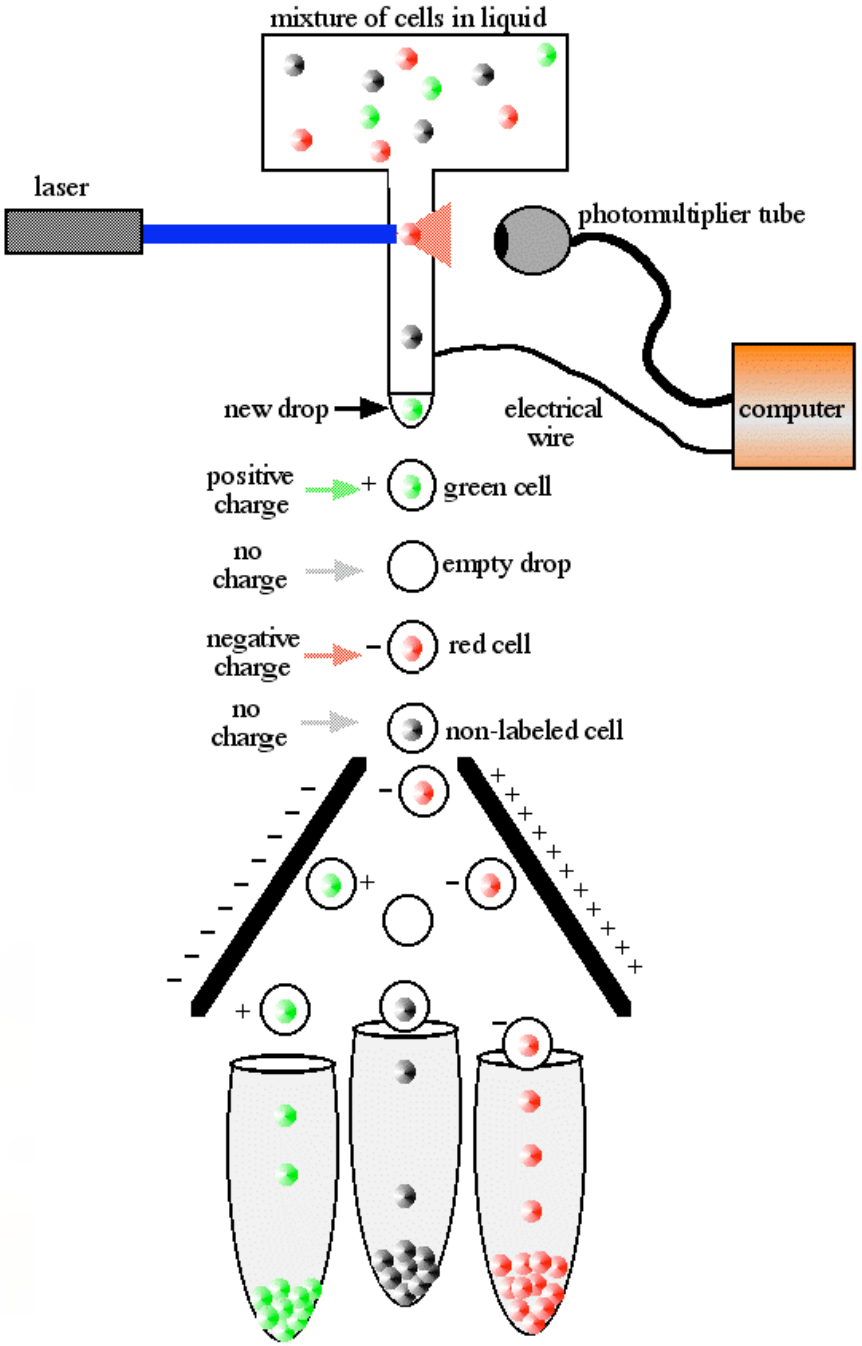
4-Immunofluorescenza (IF)

5-Analisi citofluorimetrica (Fluorescence Activated Cell Sorter: FACS)

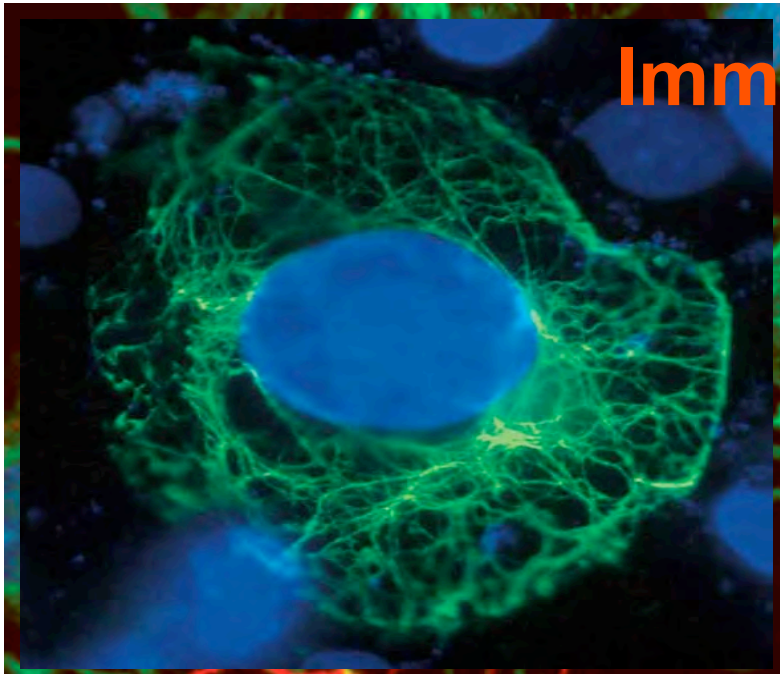
6-Immunoprecipitazione (IP)

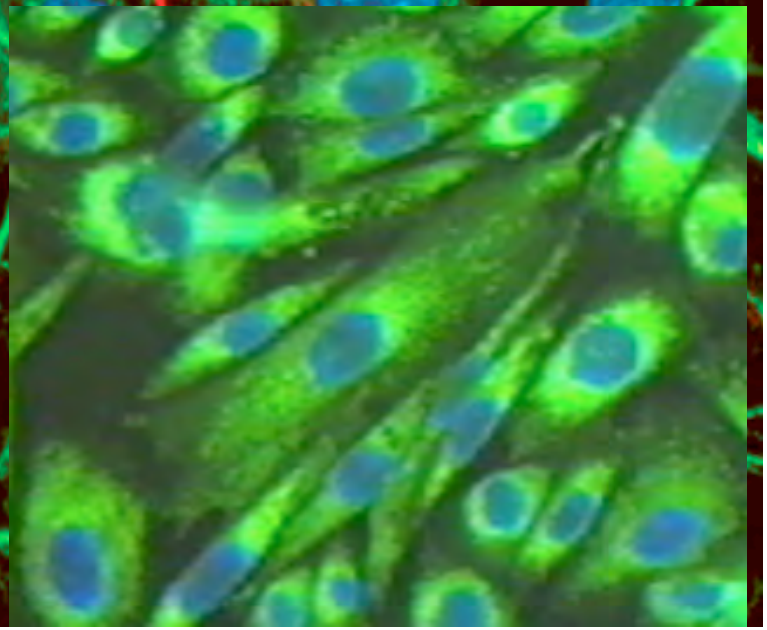
7-Western Blotting (WB)

Fluorescence-Activated Cell Sorter (FACS)

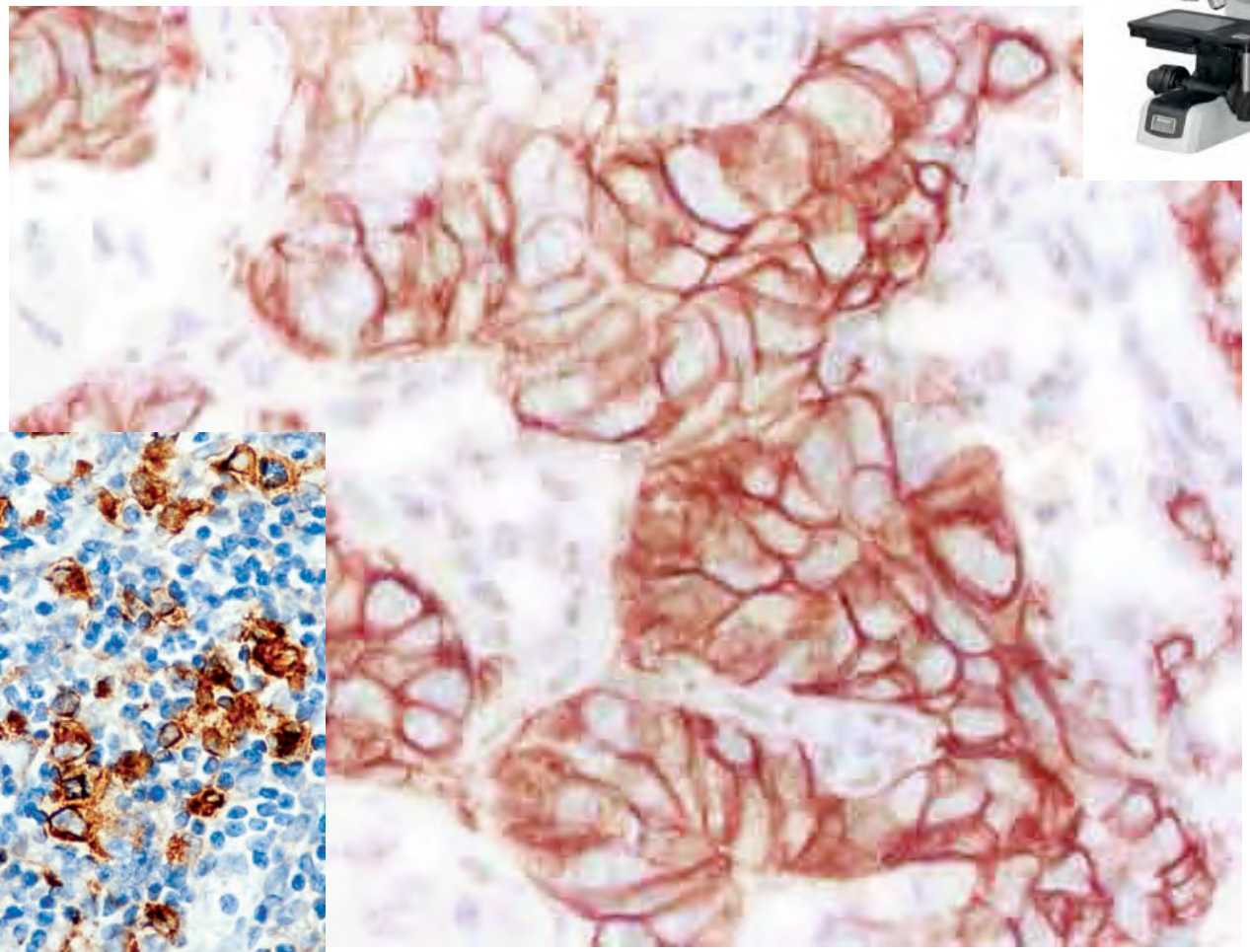


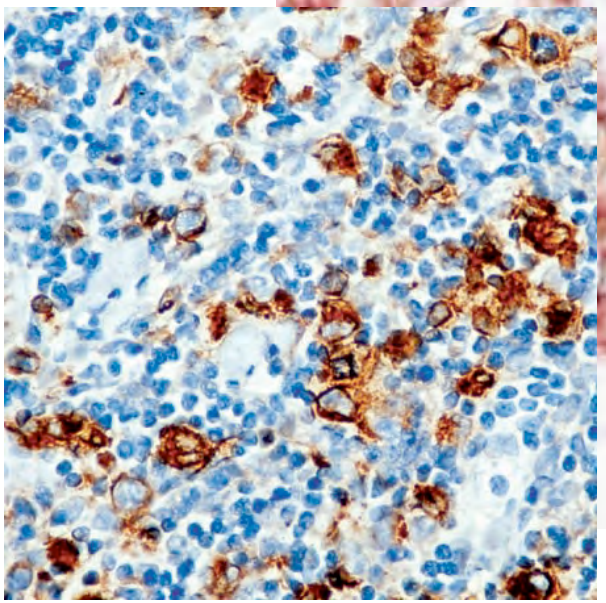
Immunofluorescence





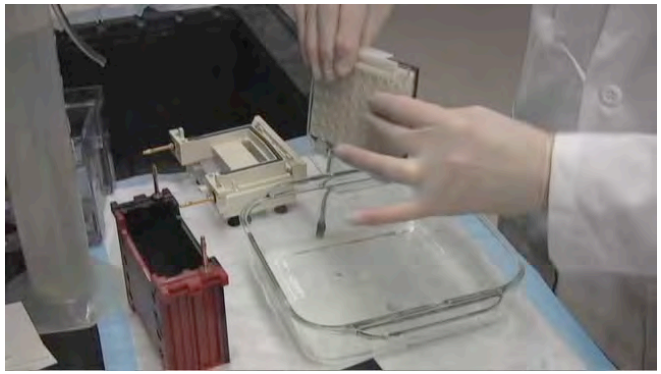
Immunohistochemistry

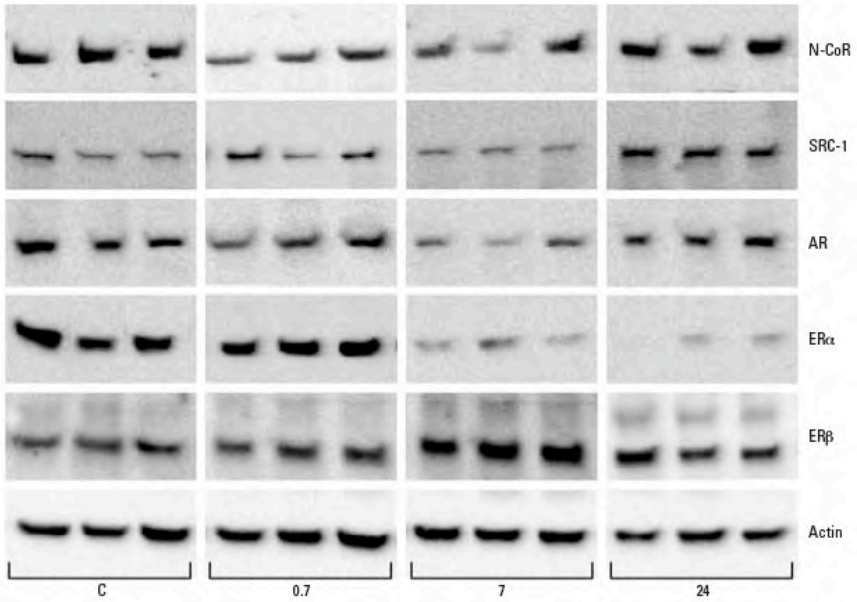




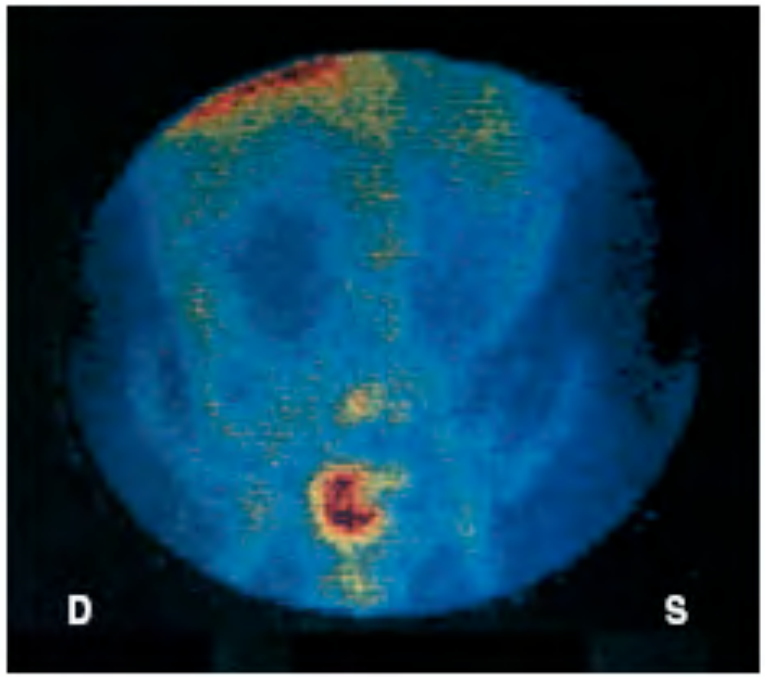
Enzyme-Linked Immunoadsorbent Assay (ELISA)

Protein quantitation by immunoblot (western blotting)





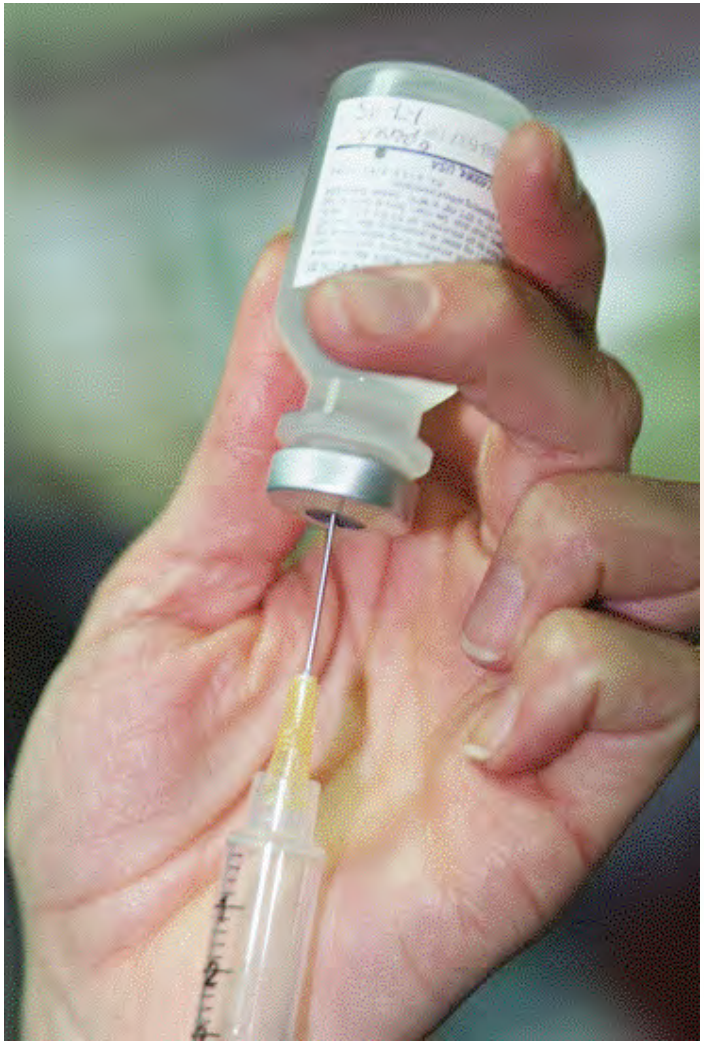
RIS = RadioImmunoScintigrafia: recidiva tumore colon-retto individuato con mAb marcato con un isotopo radioattivo, diretto contro l'antigene carcinoembrionario (CEA)

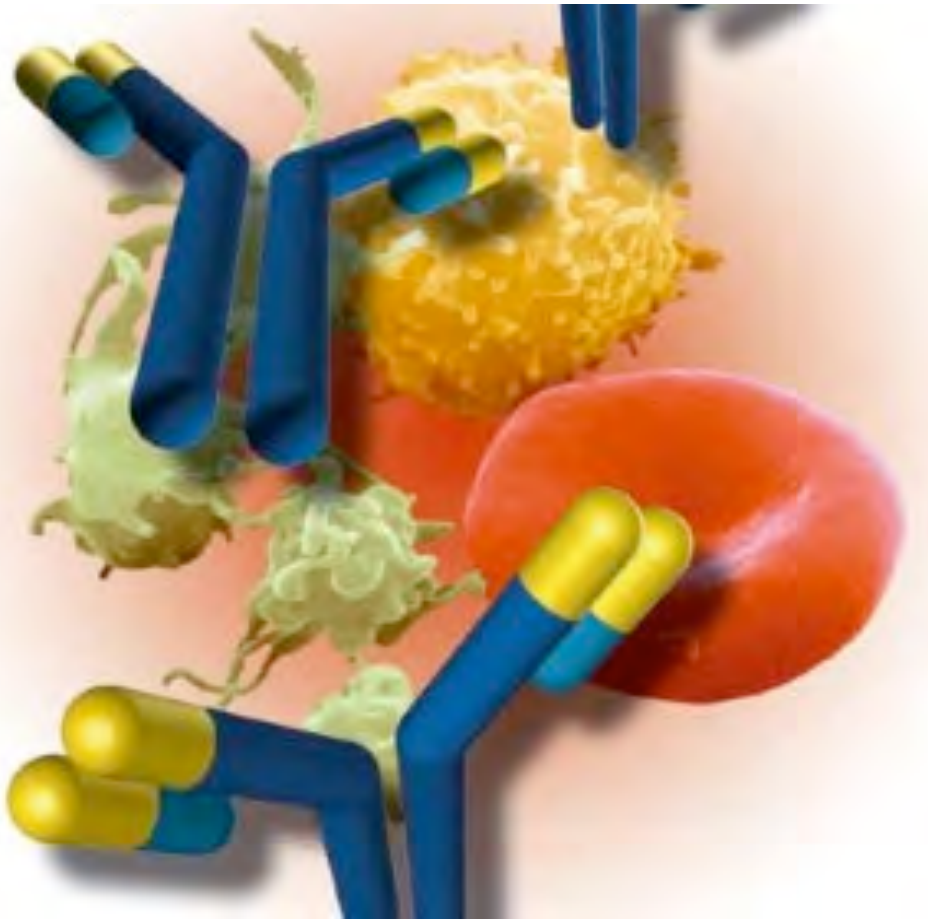


Regione pelvica

Iniezione endovena del mAb anti-CEA marcato con indio-111

Therapeutic mAbs





Murine mAbs

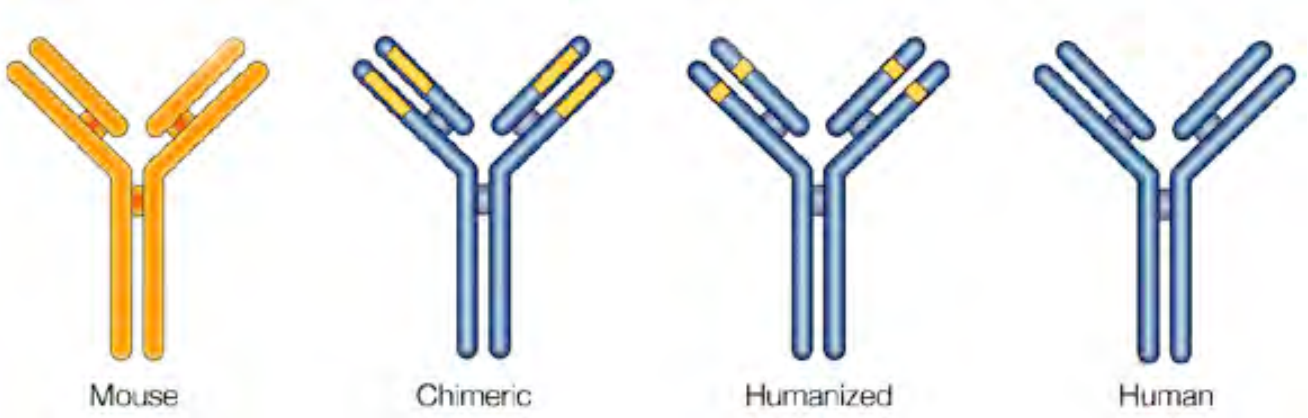
1) La somministrazione di immunoglobuline appartenenti a specie differenti induce nell'uomo una risposta immunitaria specifica denominata **HAMA** (Human Anti Mouse Antibodies), che può sia renderne inefficace l'azione che causare reazioni immunologiche anche gravi.

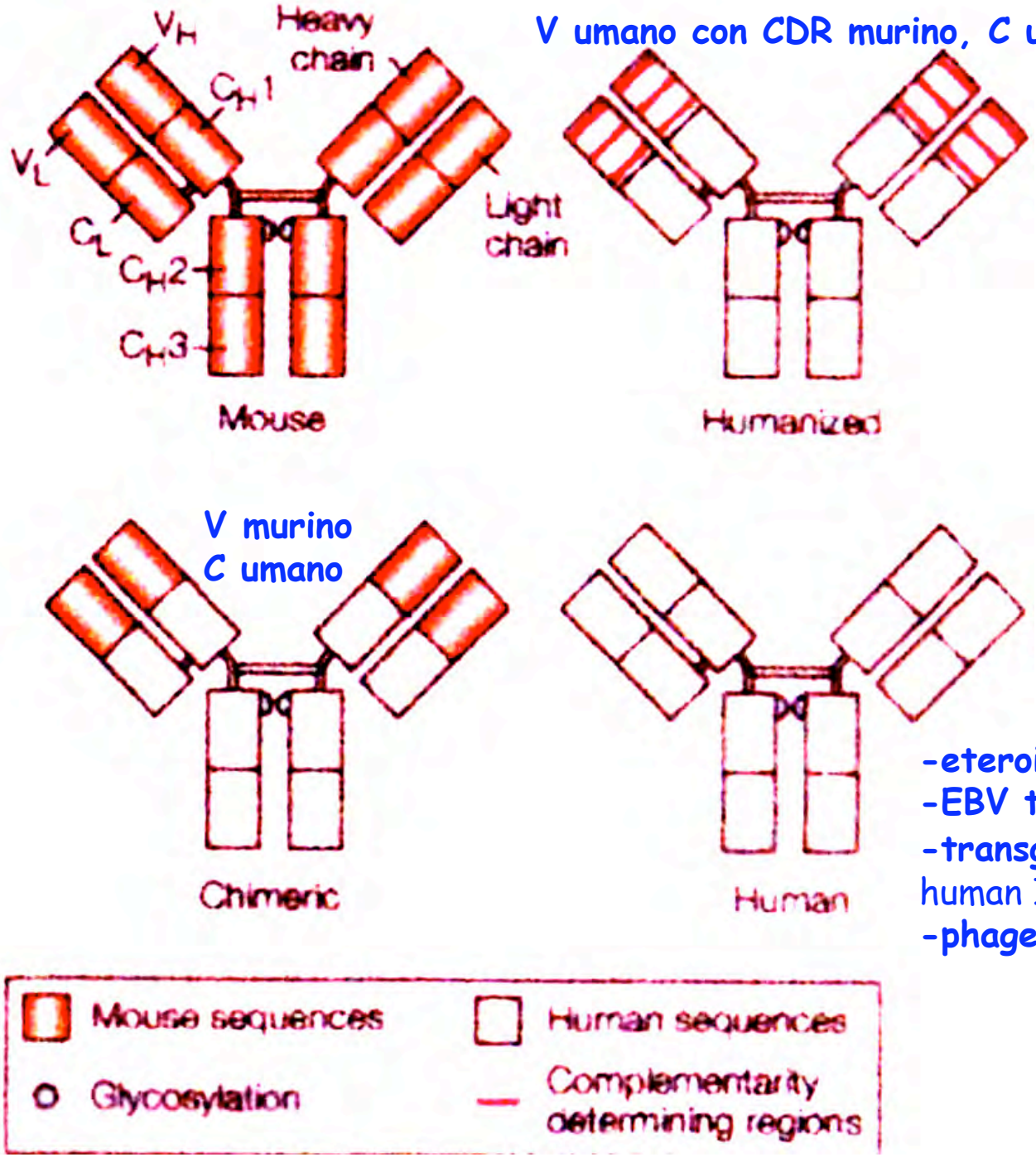
2) L'**emivita** delle IgG murine è di circa 24 h, mentre quelle umane superano i 20 giorni.

3) I mAbs murini hanno una **limitata capacità di fissare il complemento** e di interagire con cellule effettrici umane (**inefficiente ADCC**: citotossicità cellulare anticorpo mediata).

Classes of monoclonal antibodies (mAbs)

- **Murine** mAbs: classically produced in mice, completely murine molecules
- **Chimeric** mAbs: variable regions (antigen binding) from murine mAbs fused to human constant regions. Resulting mAbs are 70% human.
- **Humanized** mAbs: murine antigen-binding sequences (CDR) grafted into human antibodies. Resulting mAbs are 90% human.
- **Human** mAbs: 100% human.





-eteroibridomi uomo-topo
-EBV transformation
-transgenic mice (expressing human Ig genes)
-phage display library

HumAbs

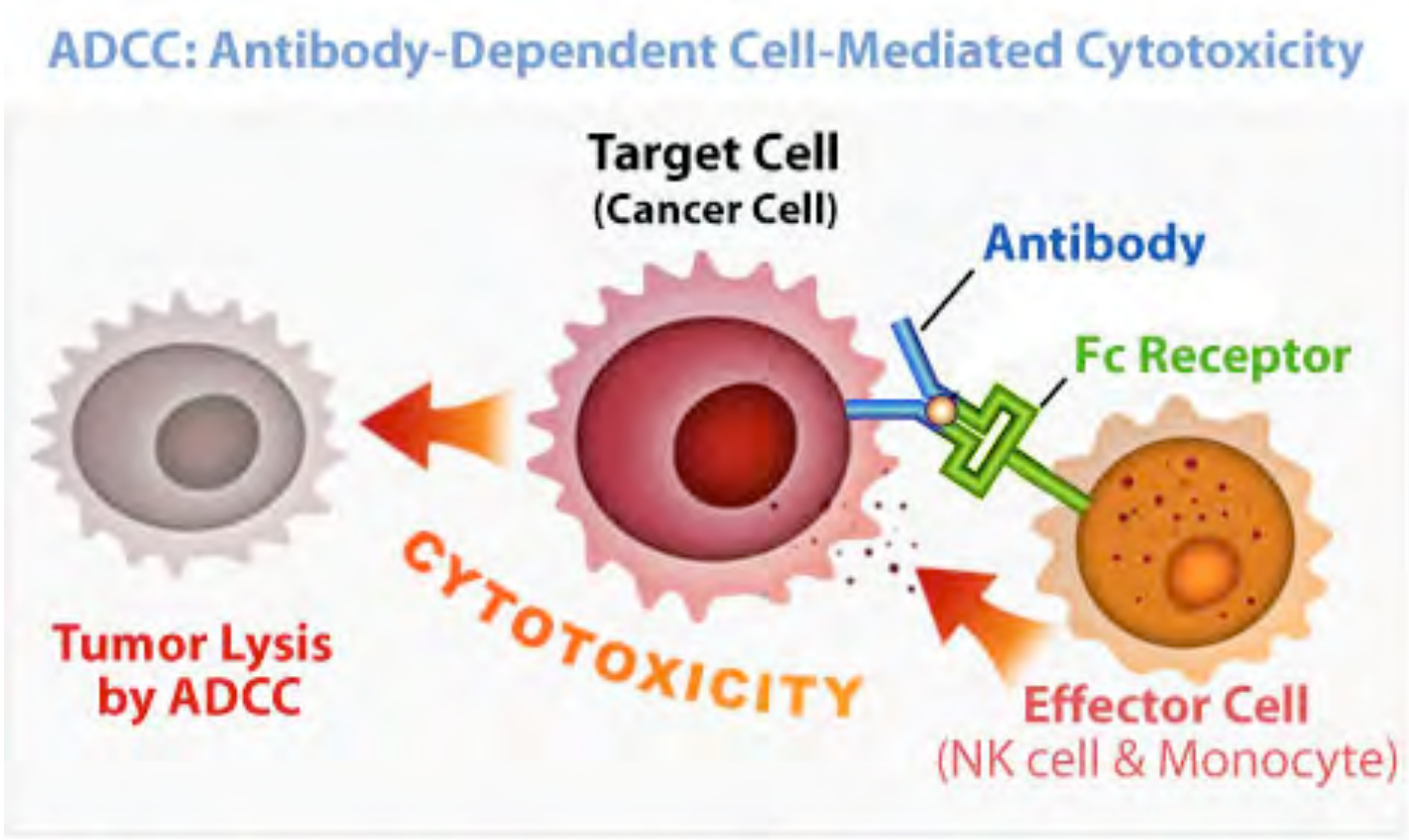
Table 1. The production of human antitumour monoclonals (current approaches)*

Immunisation status	(a) Active disease alone (b) *In vitro* immunisation or boosting (c) Active disease plus immunisation with tumour vaccine
Lymphoid tissues used	(a) Draining lymph nodes (b) Peripheral blood lymphocytes (c) Intratumour lymphocytes or spleen
Immortalisation procedures	(a) Fusion alone with human, mouse or human/mouse cell lines (b) Epstein Barr virus transformation (c) Epstein Barr virus transformation followed by fusion
Patients with tumours of the following tissues/organs	(a) Breast (b) Skin (melanoma) (c) Lung (d) Colorectum, stomach (e) Bladder, blood, brain, cervix, ovary, vulva

+CD40 system

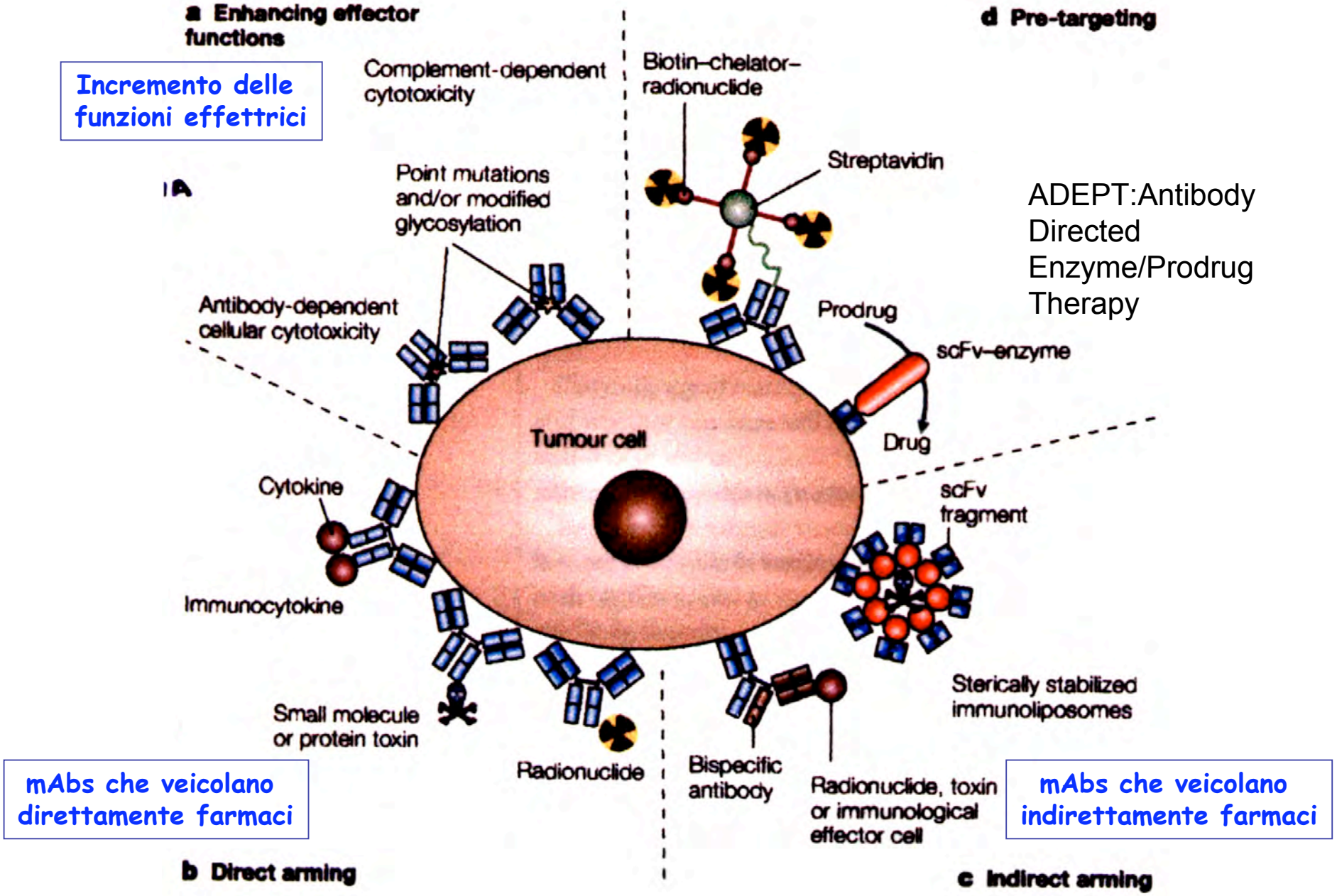
*Ranked in descending order of use to date. For further details see ref 6.

Therapeutic mAbs: enhancing effector functions



ADCC is a major mechanism for killing tumor cells by therapeutic antibodies.

Therapeutic mAbs: oncotherapy



ADEPT:Antibody Directed Enzyme/Prodrug Therapy

Problems associated with *in vivo* therapy with monoclonal antibodies

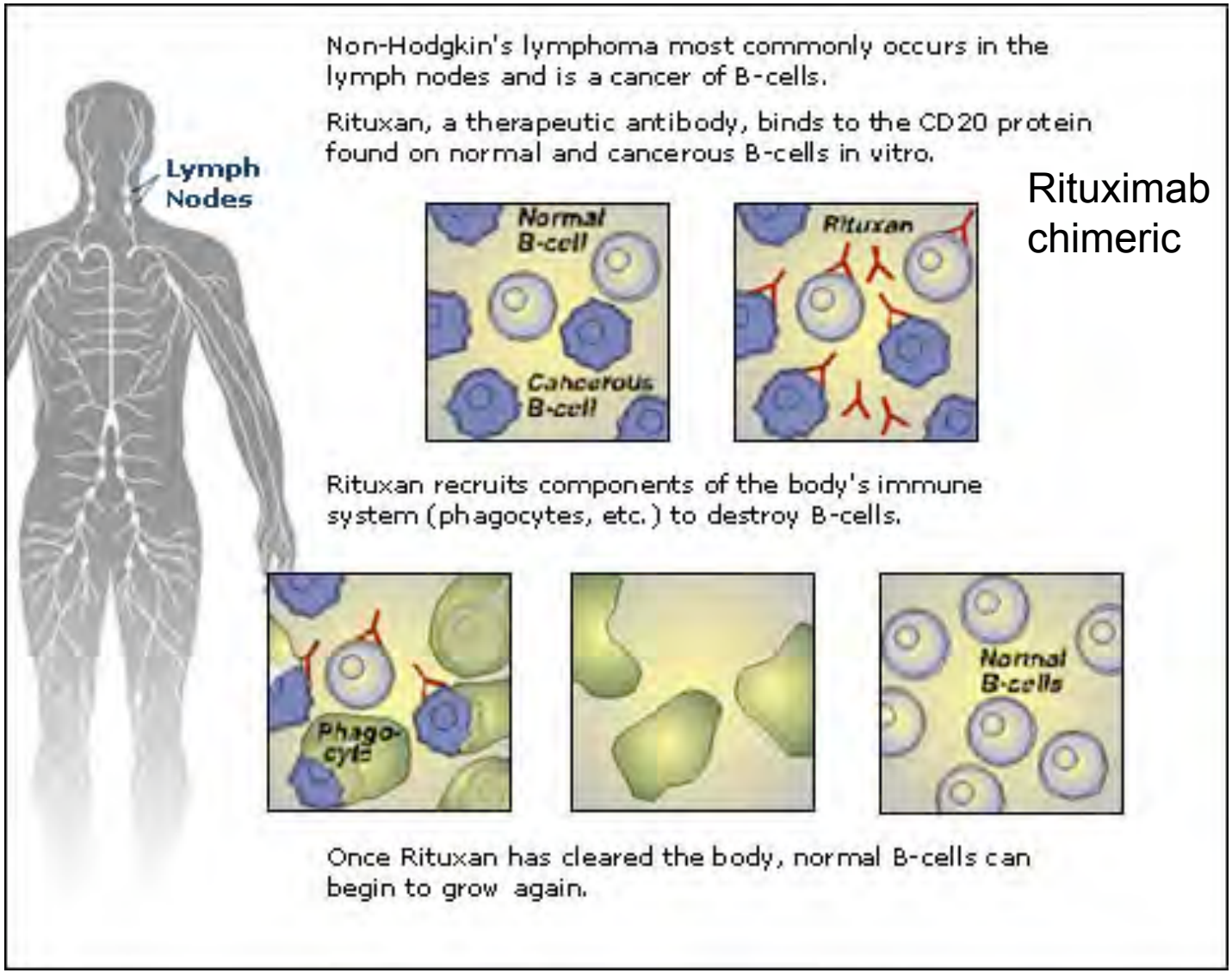
1. Circulating free antigen
2. Antigenic modulation (internalization, capping)
3. Host antibody response to the monoclonal antibody
4. Selection for cells that do not express antigen (antigen-loss variants)
5. Sanctuary sites (antibodies and/or lymphocytes or reticuloendothelial cells denied access)

Trattamento dei tumori:

Difficoltà:

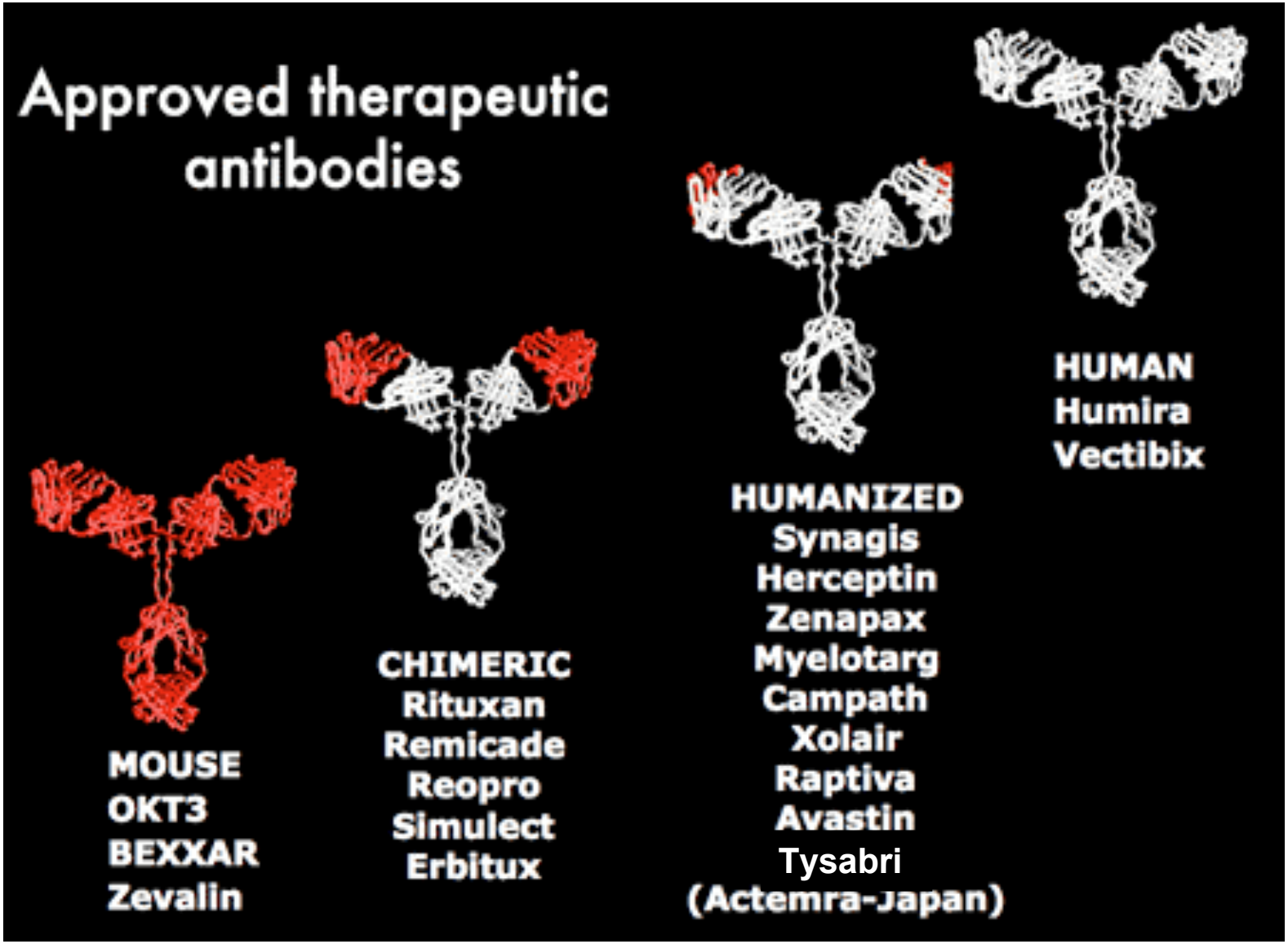
1. Limitazione della penetrazione dell'anticorpo in una massa voluminosa
2. Legame alle cellule non tumorali
3. Anticorpi sono comunque immunogenici e inducono una risposta anti-idiotipo

Therapeutic mAbs



Antigeni tumorali marcati con anticorpi monoclonali in trial terapeutici

Origine del tessuto tumorale	Tipo di antigene	Antigene	Tipo di tumore	
Linfoma/ leucemia	Antigene di differenziamento	CD5 Idiotipo CD52 (CAMPATH1)	Linfoma a cellule T Linfoma a cellule B Linfoma/leucemia a cellule T e B	Campath, humanized
	Recettore di segnale della cellula B	CD20	Linfoma non-Hodgkin a cellule B	Rituxan, chimeric
Tumori solidi	Antigeni di membrana glicoproteine Carboidrati	CEA, mucina-1 Lewis^y CA-125	Tumori epiteliali (mammella, colon, polmone) Tumori epiteliali Carcinoma ovarico	Erbitux, chimeric / Vectibrix,human
	Recettore per fattore di crescita	Recettore per fattore epidermico di crescita HER-2/ neu Recettore IL-2 Fattore di crescita vascolare endoteliale (VEGF)	Tumori mammari, del polmone, della testa e del collo Tumori mammari e ovarici Tumori delle cellule T e B Cancro colon polmone, prostata, mammella	Simulect, chimeric / Zenapax, humanized Avastin, Humanized
	Antigene dello stroma extracellulare	FAP-α Tenascina Metalloproteinasi	Tumori epiteliali Glioblastoma multiforme Tumori epiteliali	


Approved therapeutic antibodies
MOUSE
OKT3
BEXXAR
Zevalin
CHIMERIC
Rituxan
Remicade
Reopro
Simulect
Erbitux
HUMANIZED
Synagis
Herceptin
Zenapax
Myelotarg
Campath
Xolair
Raptiva
Avastin
Tysabri
(Actemra-Japan)
HUMAN
Humira
Vectibix

FDA-approved mAbs for therapeutic use

Company	drug	molecule	date	type	antibody
Ortho (J&J)	Ortho-OKT3	muromonab-CD3	1992	murine	anti-CD3
Immunomedics	CEA-Scan	arcitumomab	1996	murine	anti-CEA Fab
Centocor (J&J)	Myoscint	imciromab pentetate	1996	murine	anti-myosin Fab
Cytogen (EUSA Pharma)	Prostascint	capromab pendetide	1996	murine	anti-PSMA
Genentech (Roche)/Biogen	Rituxan	rituximab	1997	chimeric	anti-CD20
Roche	Zenapax	daclizumab	1997	humanized	anti-CD25
Novartis	Simulect	basiliximab	1998	chimeric	anti-CD25
Genentech (Roche)	Herceptin	trastuzumab	1998	humanized	anti-HER2
Medimmune (AstraZeneca)	Synagis	palivizumab	1998	humanized	anti-RSV
Centocor (J&J)/Merck	Remicade	infliximab	1998	chimeric	anti-TNFalpha
Wyeth (Pfizer)	Mylotarg	gemtuzumab ozogam	2000	humanized	anti-CD33
Ilex Pharma	Campath	alemtuzumab	2001	humanized	anti-CD52
Biogen Idec	Zevalin	ibritumomab tiuxetan	2002	murine	anti-CD20
Abbot	Humira	adalimumab	2002	human	anti-TNFalpha
Genentech (Roche)	Raptiva	efalizumab	2003	humanized	anti-CD11a
GlaxoSmithKline	Bexxar	tositumumab	2003	murine	anti-CD20
Centocor (J&J)	Reopro	abciximab	2003	chimeric	anti-GP IIb/IIIa Fab
Genentech (Roche)	Xolair	omalizumab	2003	humanized	anti-IgE
Biogen Idec	Tysabri	natalizumab	2004	humanized	anti-alpha4 integrin
Imclone (Eli Lilly)	Erbitux	cetuximab	2004	chimeric	anti-EGFR
Centocor (J&J)/Merck	Avastin	bevacizumab	2004	humanized	anti-VEGF-A
Amgen	Vectibix	panitumumab	2006	human	anti-EGFR
Genentech (Roche)	Lucentis	ranibizumab	2006	humanized	anti-VEGF-A fragm
Alexion	Soliris	eculizumab	2007	humanized	anti-complement C5
UCB	Cimzia	certolizumab pegol	2008	humanized	anti-TNFalpha

Main category	Type	Application	Trade name	Mechanism/Target	Mode
Anti-inflammatory	infliximab[23]	• rheumatoid arthritis • Crohn's disease • Ulcerative Colitis	Remicade 1998	inhibits TNF-α	chimeric
	adalimumab	• rheumatoid arthritis • Crohn's disease • Ulcerative Colitis	Humira 2002	inhibits TNF-α	human
	etanercept[23]	• rheumatoid arthritis	Enbrel	Contains decoy TNF receptor	fusion protein
	basiliximab[23]	• Acute rejection of kidney transplants	Simulect	inhibits IL-2 on activated T cells	chimeric
	daclizumab[23]	• Acute rejection of kidney transplants	Zenapax	inhibits IL-2 on activated T cells	humanized
	omalizumab	• moderate-to-severe allergic asthma	Xolair	inhibits human immunoglobulin E (IgE)	humanized
Anti-cancer	gemtuzumab[23]	• relapsed acute myeloid leukaemia	Mylotarg	targets myeloid cell surface antigen CD33 on leukemia cells	humanized
	alemtuzumab[23]	• B cell leukemia	Campath	targets an antigen CD52 on T- and B-lymphocytes	humanized
	rituximab[23]	• non-Hodgkin's lymphoma	Rituxan	targets phosphoprotein CD20 on B lymphocytes	chimeric
	trastuzumab	• breast cancer with HER2/neu overexpression	Herceptin	targets the HER2/neu (erbB2) receptor	humanized
	nimotuzumab	• Approved in squamous cell carcinomas, Glioma • Clinical trials for other indications underway	Theracim	EGFR inhibitor	Humanized
	cetuximab	• Approved in squamous cell carcinomas, colorectal carcinoma	Erbitux	EGFR inhibitor	Chimeric
	bevacizumab	• Anti-angiogenic cancer therapy	Avastin	inhibits VEGF	humanized
Other	palivizumab[23]	• RSV infections in children	Synagis	inhibits an RSV fusion (F) protein	humanized
	abciximab[23]	• Prevent coagulation in coronary angioplasty	Reopro	inhibits the receptor GpIIb/IIIa on platelets	chimeric

Agenti terapeutici per l'autoimmunità umana				
Bersaglio	**Agente terapeutico**	**Malattia**	**Esito della malattia**	**Svantaggi**
Integrina	anticorpo monoclonale (mAb) $\alpha_4\beta_1$ integrina specifico Natalizumab, humanized	Sclerosi multipla (MS) recidivante/remittente Artrite reumatoide (RA) Malattia infiammatoria dell'intestino	Riduzione della frequenza delle recidive; ritardo della progressione della malattia	Aumento del rischio di infezione, encefalopatia progressiva multifocale
Cellule B	mAb CD20-specifico Rituximab, chimeric	RA Lupus eritematoso sistemico (LES) MS	Miglioramento dell'artrite, possibile nel LES	Aumento del rischio di infezione
HMG-coenzima A reduttasi	Statine	MS	Riduzione dell'attività della malattia	Epatossicità; rabdomiolisi
Cellule T	mAb CD3-specifico	Diabete mellito tipo 1	Riduzione dell'uso dell'insulina	Aumento del rischio di infezione
	Proteina di fusione CTLA4-immunoglobulina	RA Psoriasi MS	Miglioramento dell'artrite	

Agenti terapeutici per l'autoimmunità umana				
Bersaglio	**Agente terapeutico**	**Malattia**	**Esito della malattia**	**Svantaggi**
Citochine	mAb TNF-specifico e proteina solubile di fusione di TNFR Remicade, Enbrel	RA Malattia di Crohn Artrite psoriasica Spondilite anchilosante	Miglioramento dell'invalidità; riparazione dell'articolazione nell'artrite	Aumento del rischio di tubercolosi e di altre infezioni; lieve aumento del rischio di linfoma
	Antagonista del recettore IL-1	RA	Miglioramento dell'invalidità	Scarsa efficacia
	mAb IL-15-specifico	RA	L'invalidità può migliorare	Aumento del rischio di infezioni opportunistiche
	mAb recettore-specifico di IL-6	RA	Diminuzione dell'attività della malattia	Aumento del rischio di infezioni opportunistiche
	Interferoni tipo 1	MS recidivante/remittente	Riduzione della frequenza delle recidive	Tossicità epatica; è comune una sindrome simile all'influenza

FDA-approved mAbs for therapeutic use
annual sales

Million $

Company	drug	antibody	sales
Centocor (J&J)/Merck	Remicade	anti-TNFalpha	7,000
Abbot	Humira	anti-TNFalpha	4,500
Genentech (Roche)/Biogen	Rituxan	anti-CD20	2,900
Centocor (J&J)/Merck	Avastin	anti-VEGF-A	2,900
Genentech (Roche)	Herceptin	anti-HER2	1,800
Imclone (Eli Lilly)	Erbitux	anti-EGFR	1,700
Genentech (Roche)	Lucentis	anti-VEGF-A fragm	900
Biogen Idec	Tysabri	anti-alpha4 integrin	600
Genentech (Roche)	Xolair	anti-IgE	500