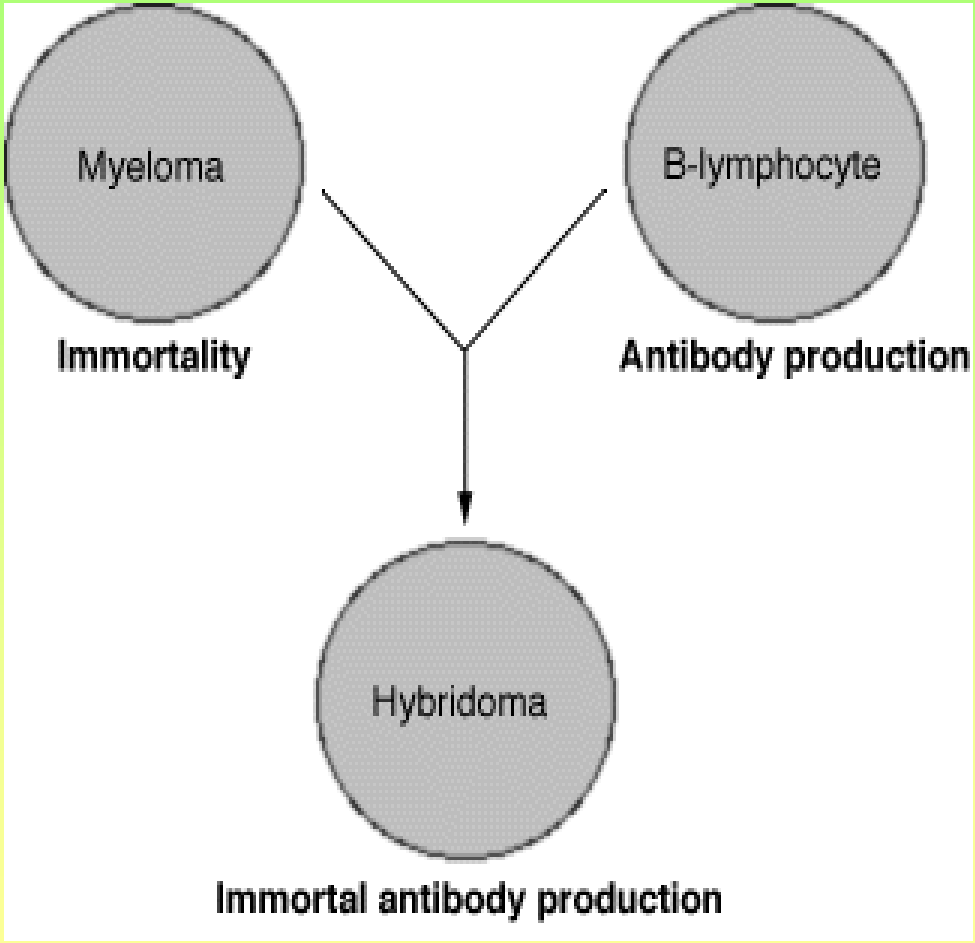
Medical Biotechnology 2024'

Biological therapies

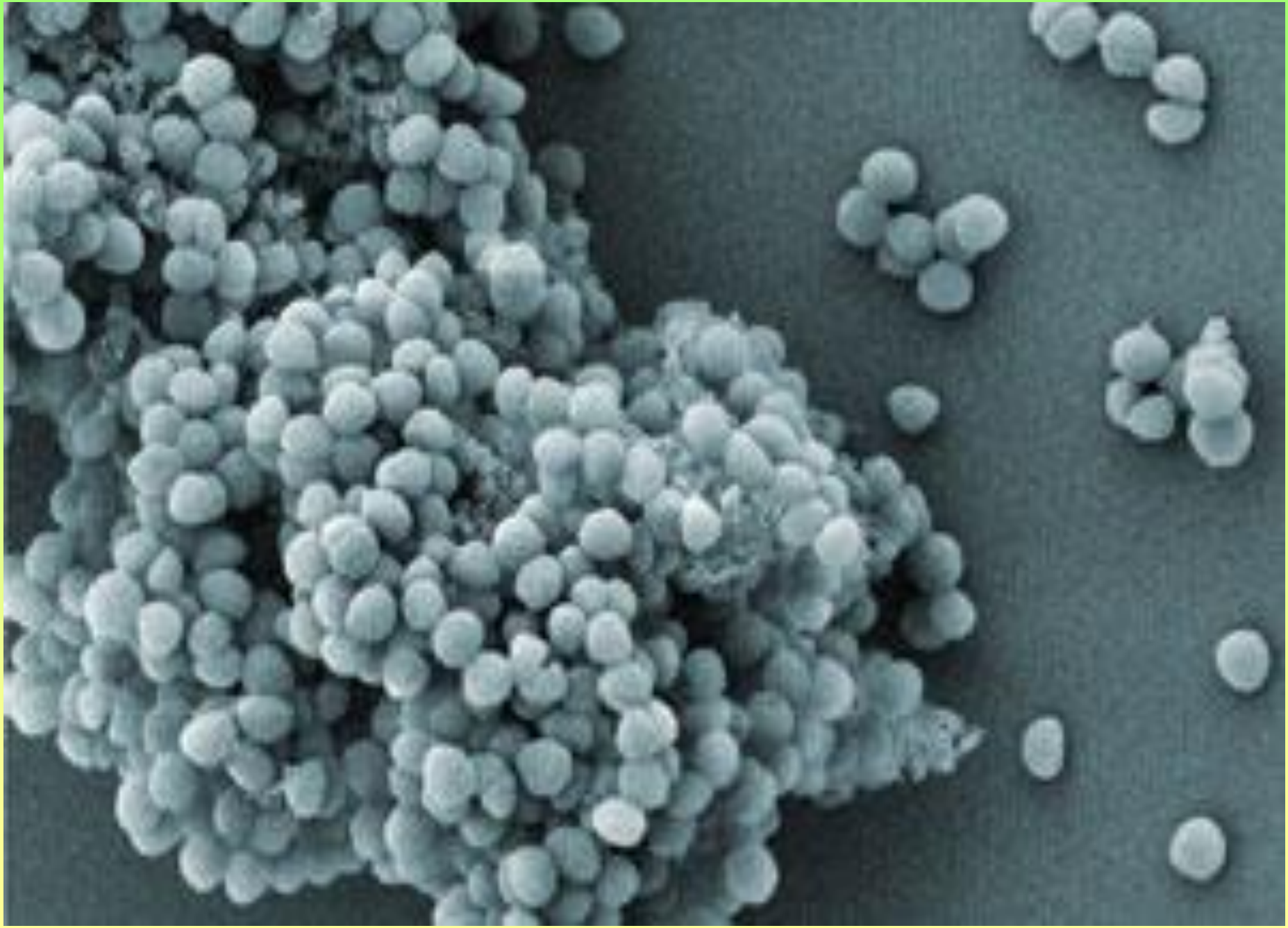
Lecture 11 - 12th

Monoclonal antibody therapy II.

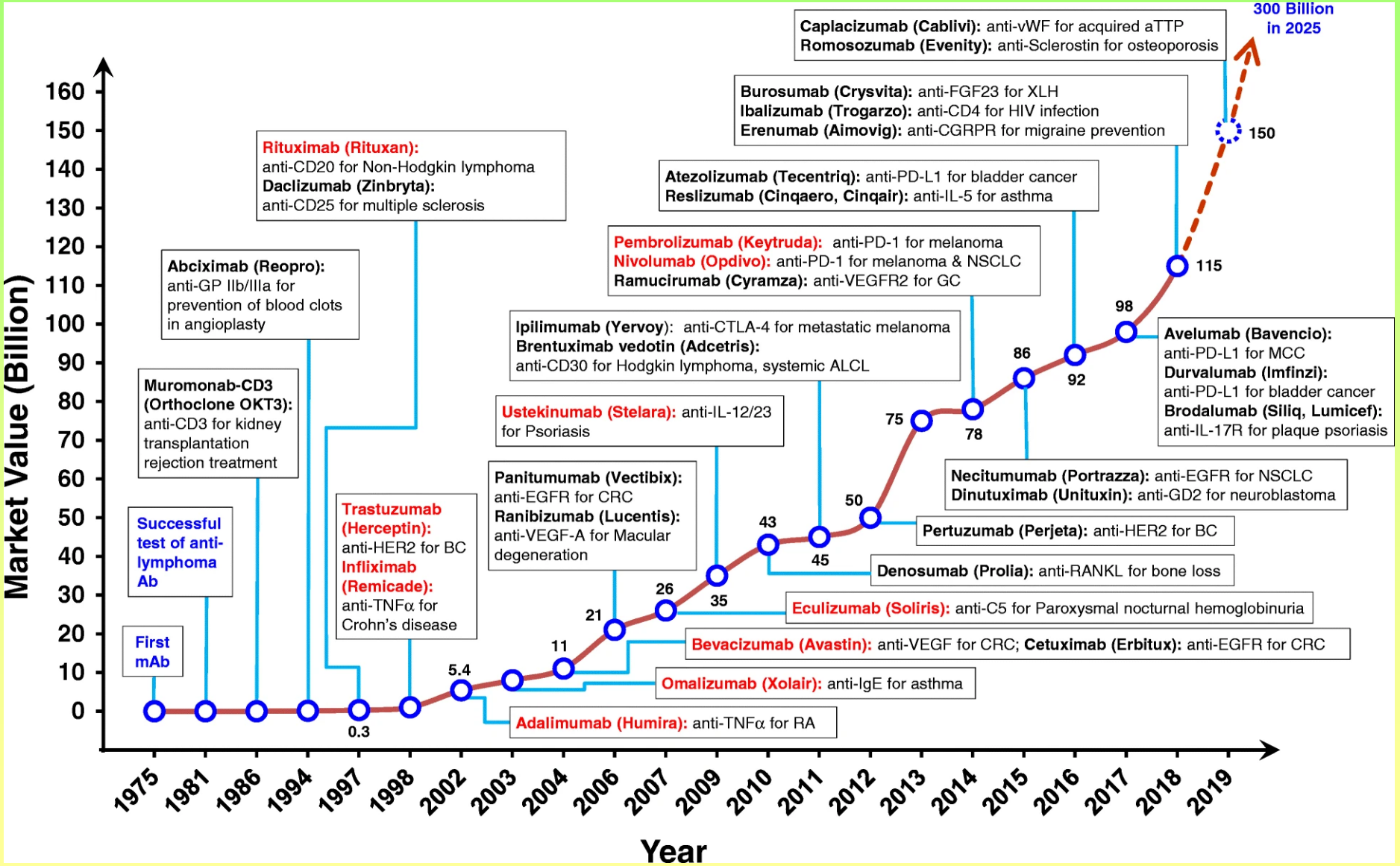
Hybridoma


Myeloma
B-lymphocyte
Immortality
Antibody production
Hybridoma
Immortal antibody production

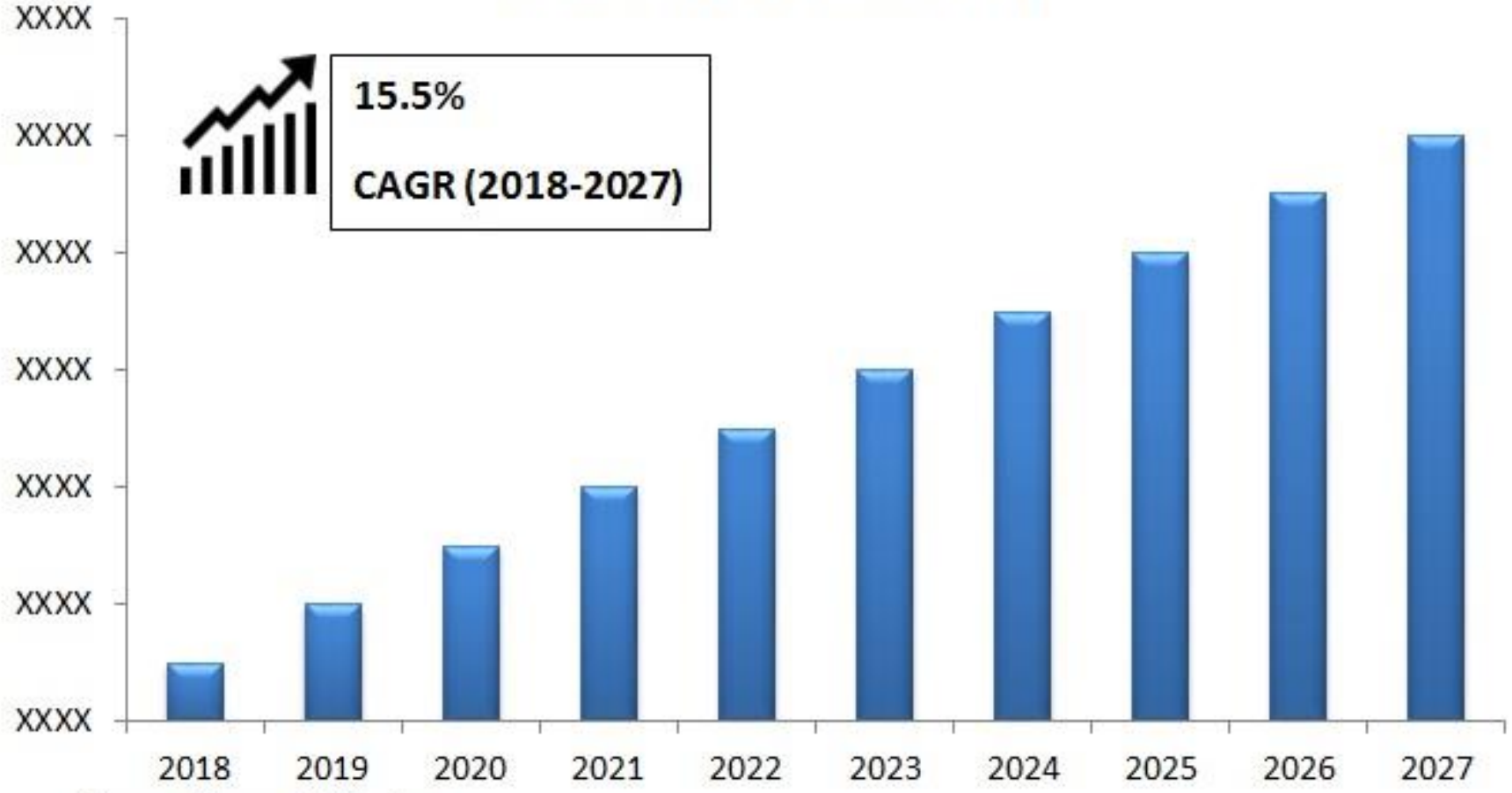

SCIENCE PHOTO LIBRARY



Development of therapeutic monoclonal antibodies for treatment of diseases

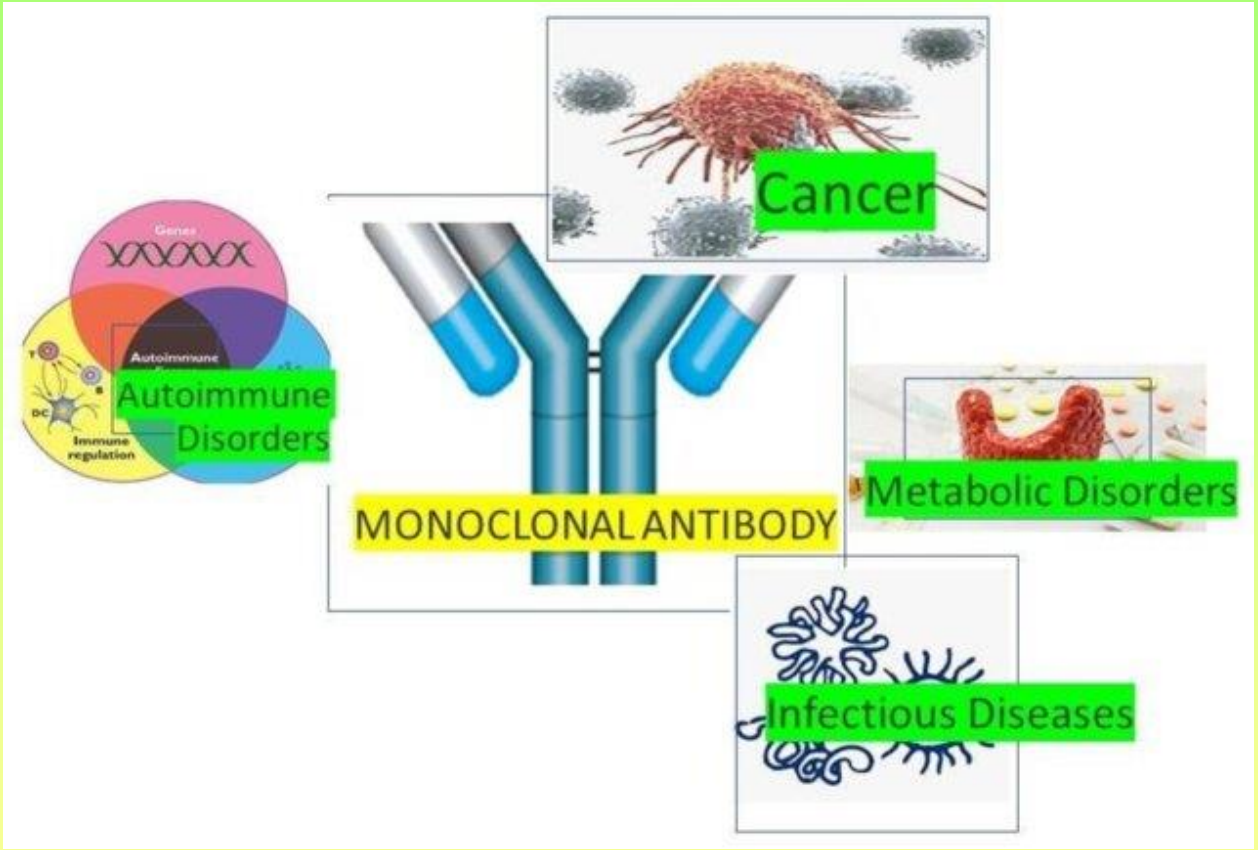


Global Monoclonal Antibodies Market Size During The Forecast Period (2018 - 2027)

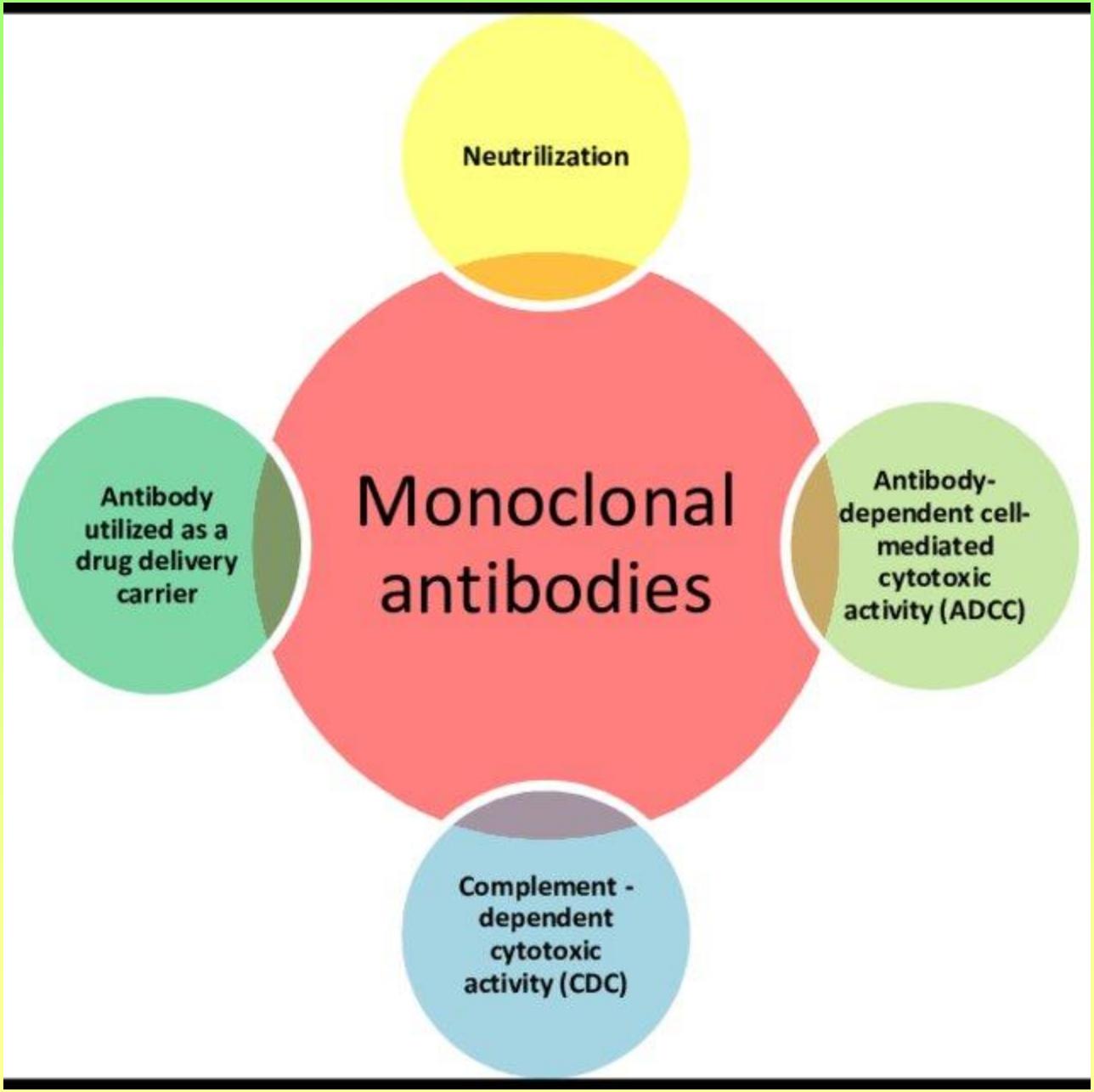


Source: Research Nester

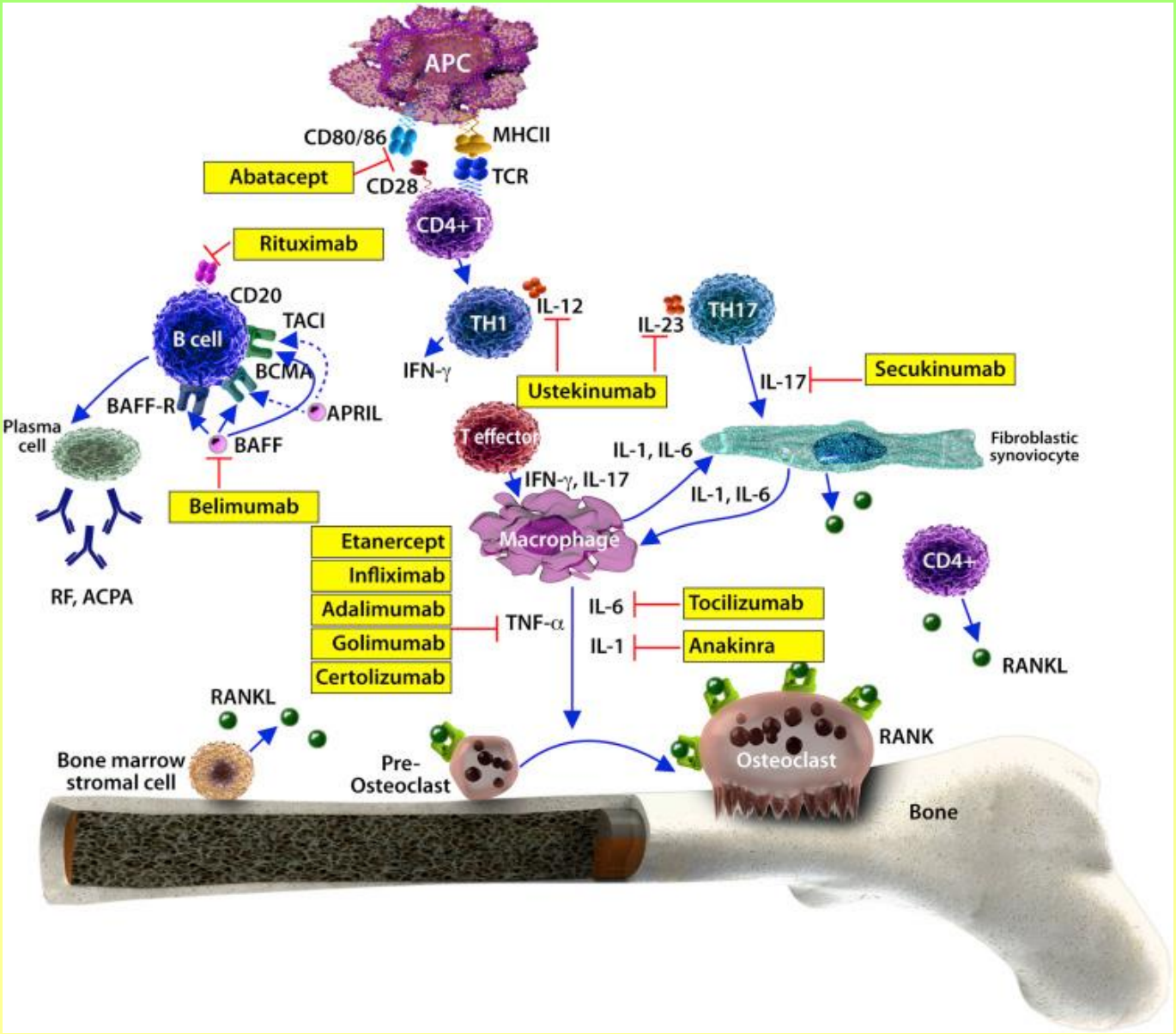
Main fields of mab therapy



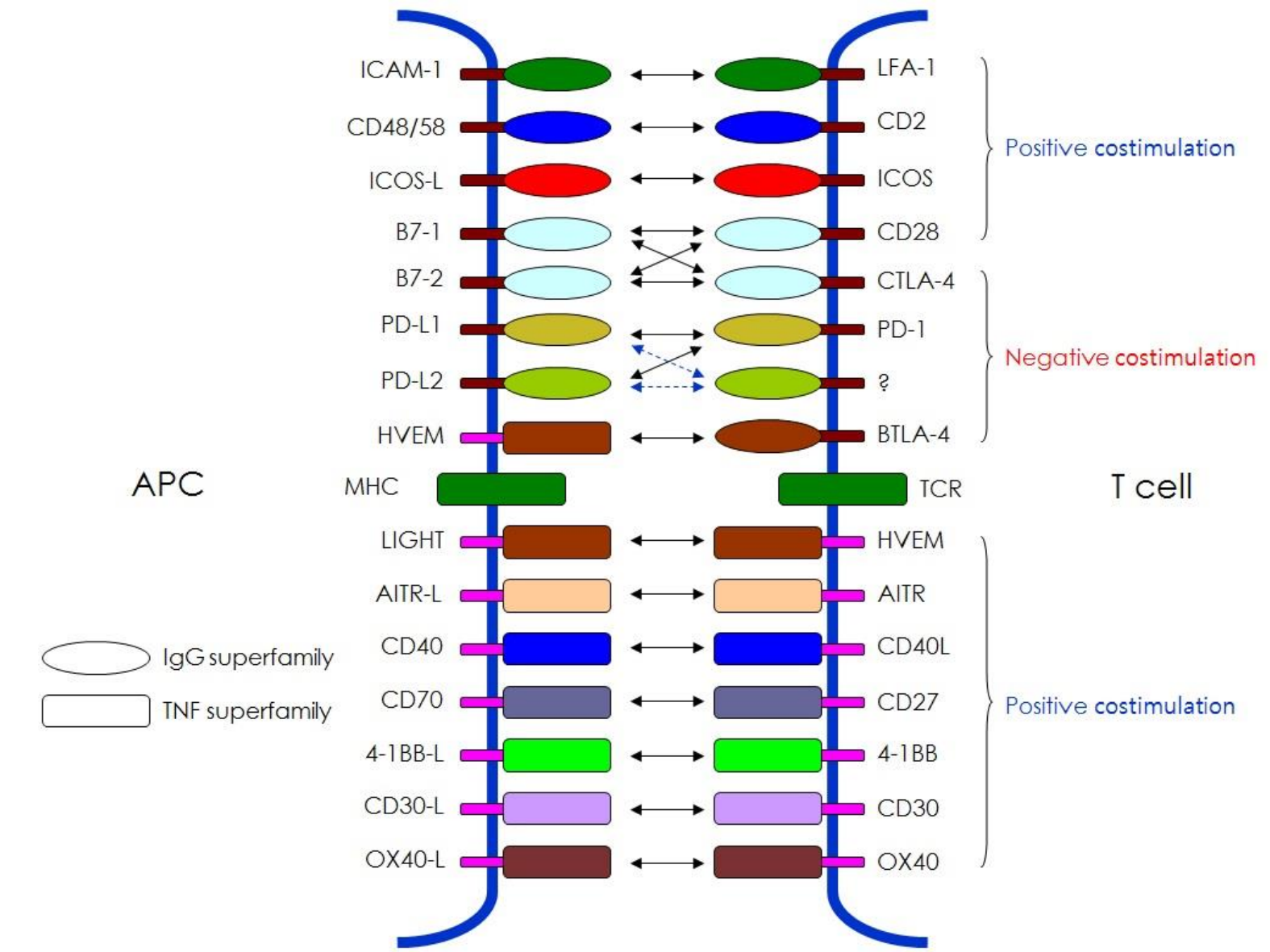
- Cancer therapy
- Autoimmune and chronic inflammatory diseases
- Organ transplantation


Neutrilization
Antibody utilized as a drug delivery carrier
Monoclonal antibodies
Antibody-dependent cell-mediated cytotoxic activity (ADCC)
Complement - dependent cytotoxic activity (CDC)

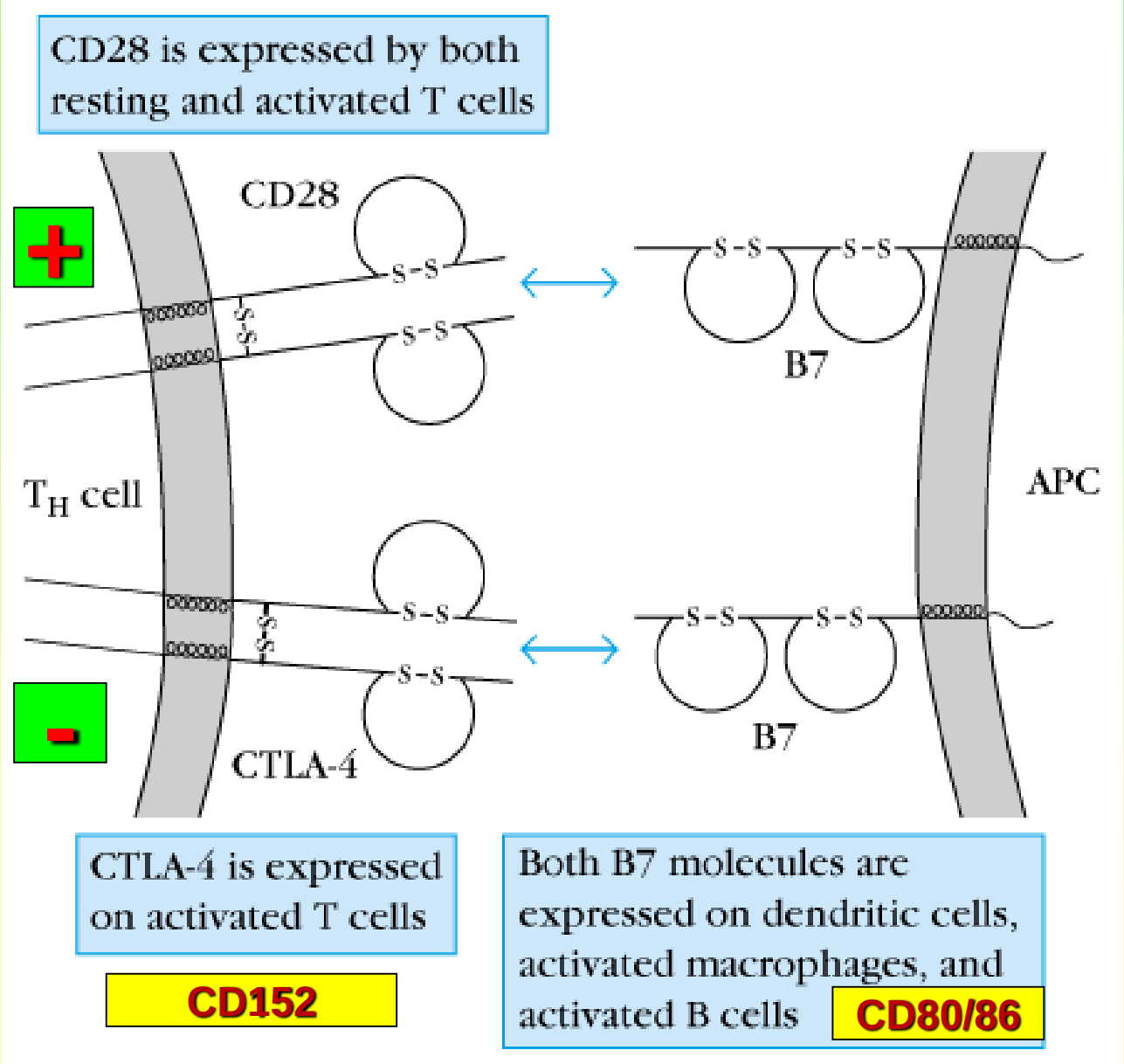
Biotherapeutical agents for rheumatic diseases and their targets



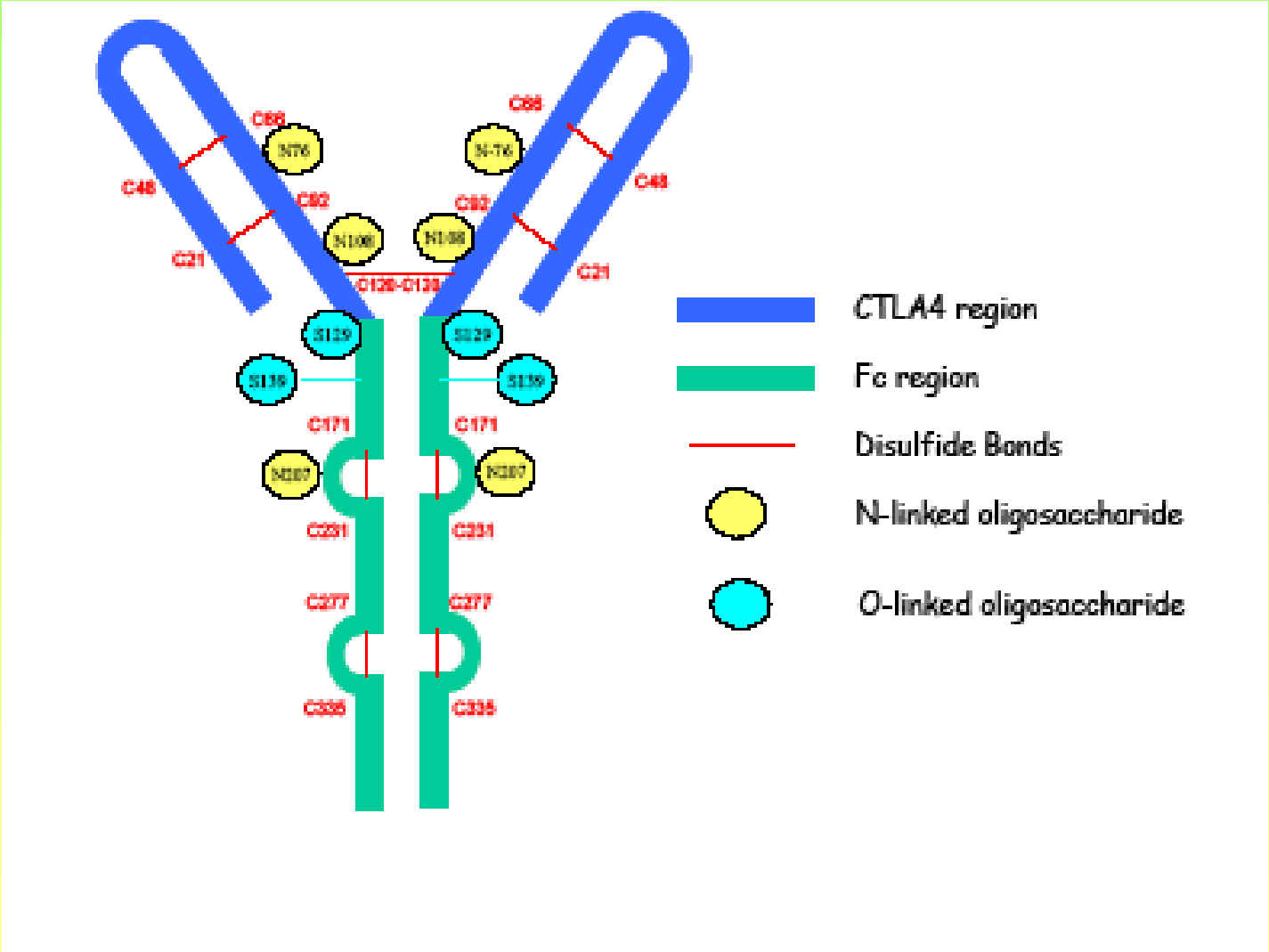
Co-stimulatory molecules in APCs and T cells

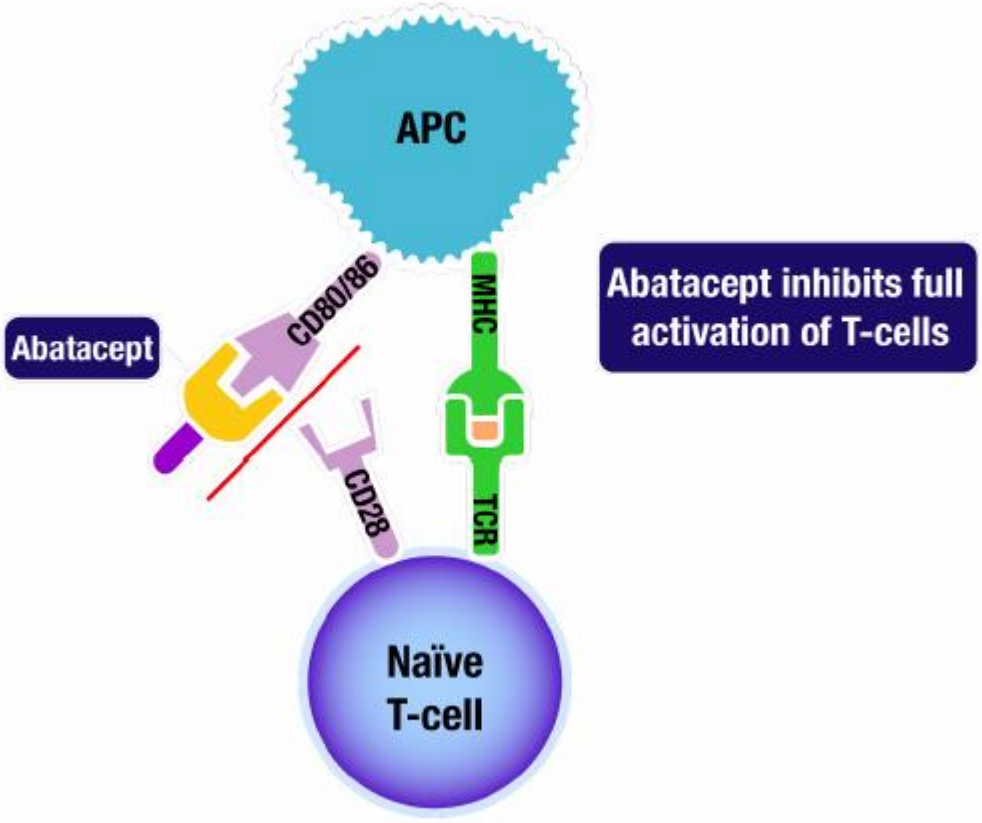


T cell activation and bolcking

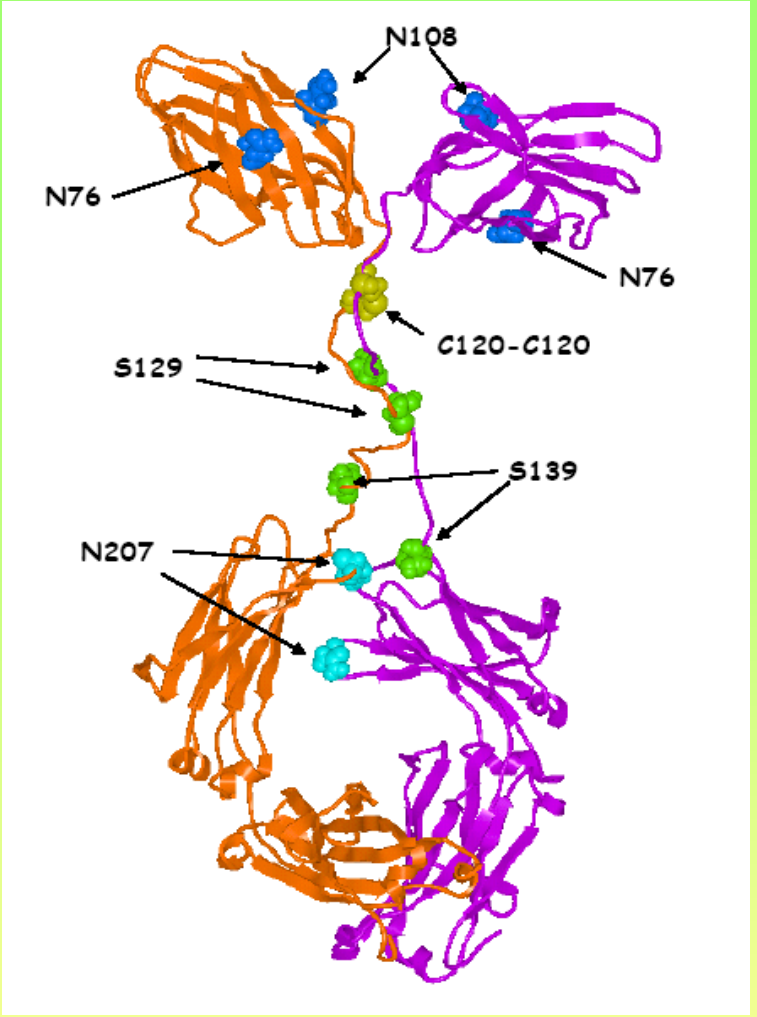


Structural model of abatacept (CTLA-4 – IgG Fc fusion protein)





APC: Antigen Presenting Cells
TCR: T-Cell Receptor
MHC: Major Histocompatibility Complex



Trade Name: **Orencia** Generic Name (USAN, INN, BAN and JAN): **Abatacept** Synonyms: CTLA4-Ig, Descriptive Name: 1-25-oncostatin M (human precursor) fusion protein with CTLA-4 (antigen) (human) fusion protein with immunoglobulin G1 (human heavy chain fragment) Laboratory Code: BMS-188667-01 (also referred to as BMS-188667) CAS Registry Number: 332348-12-6 WHO Number: 8495

Biological therapy for cancer

- Biological therapy for cancer is used in the treatment of many types of cancer to prevent or slow tumor growth and to prevent the spread of cancer.
- Therapeutic monoclonal antibodies

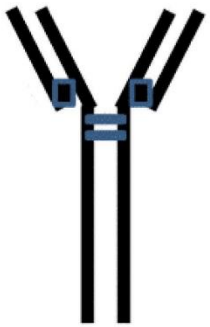
  - kill cancer cells directly

  - induce the immune system to recognize and kill cancer cells.

Monoclonal Antibodies in Cancer Therapy

Clinical Uses

Direct Tumor Targeted
Antibody-Drug Conjugates
Microenvironment Targeted
BiTEs
Immune Checkpoint Inhibitors



Effector Mechanisms of Targeted mAbs

Receptor Blocking
Ligand Blocking
ADCC
CDC
ADCP

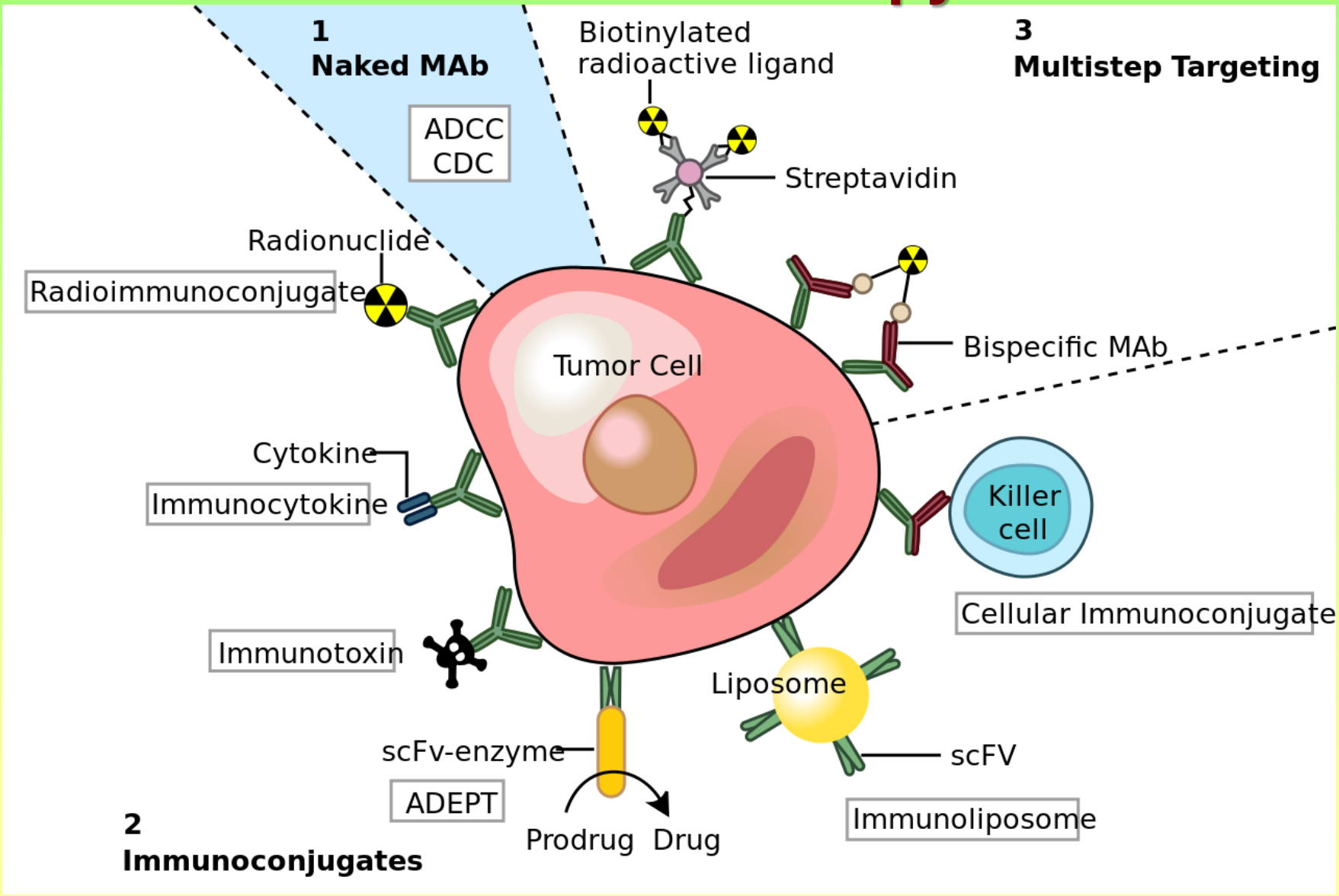
Mechanisms of Resistance

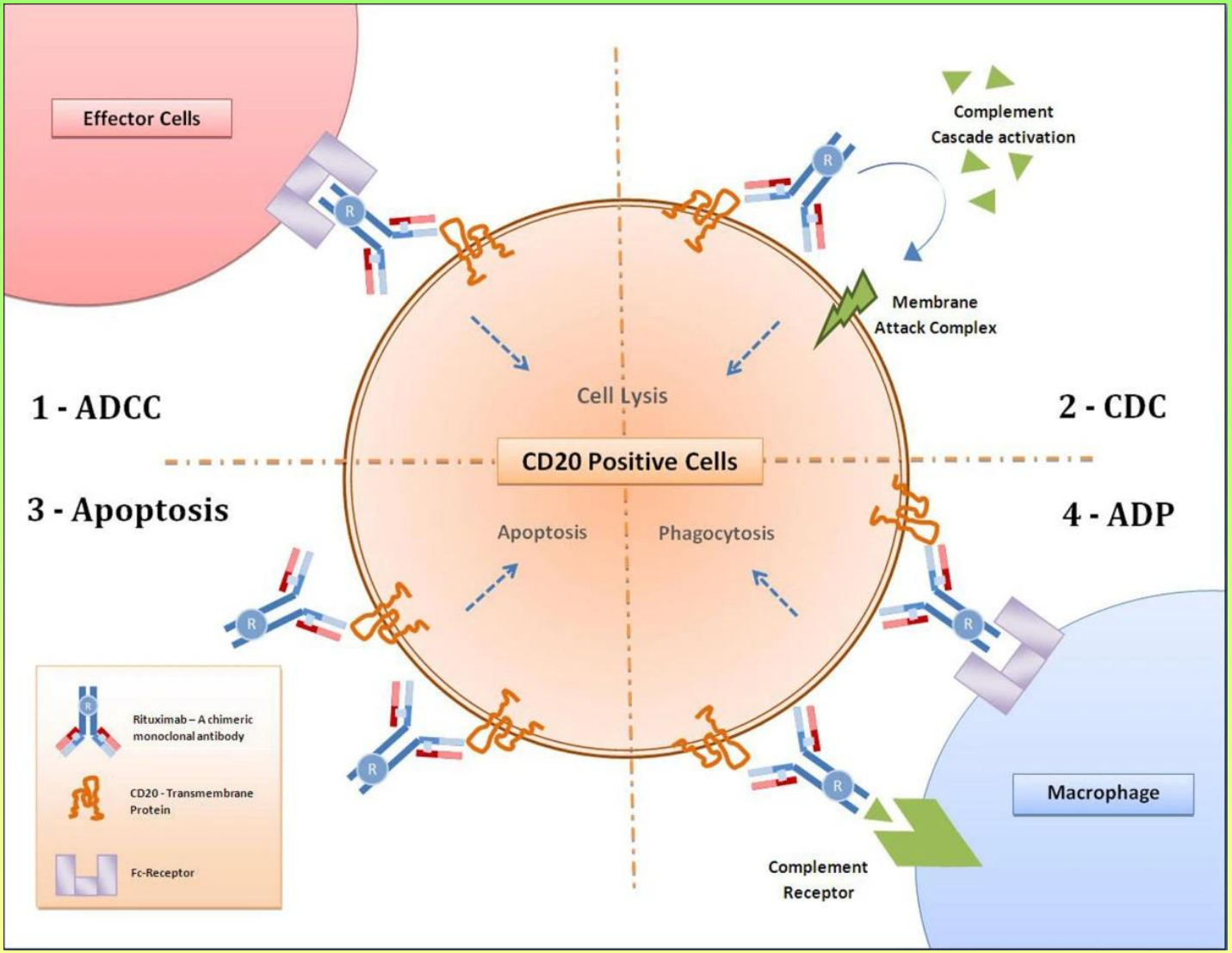
Mutations or Loss of Antibody Target
Alternative Growth/Survival Signaling
Epithelial to Mesenchymal Transition
Impaired Effector Cell Responses

Targeted antigens in tumor patients

Tumor tissue origin	Type of antigen	Antigen	Tumor type
Lymphoma/ leukemia	Differentiation antigen	CD5 Idiotype CAMPATH-1 (CDw52)	T-cell lymphoma B-cell lymphoma T- and B-cell lymphoma
	B-cell signaling receptor	CD20	Non-Hodgkin's B-cell lymphoma
Solid tumors	Cell-surface antigens Glycoprotein Carbohydrate	CEA, mucin-1 Lewis^y CA-125	Epithelial tumors (breast, colon, lung) Epithelial tumors Ovarian carcinoma
	Growth factor receptor	Epidermal growth factor receptor p185^{HER2} IL-2 receptor	Lung, breast, head, and neck tumors Breast, ovarian tumors T- and B-cell tumors
	Stromal extracellular antigen	FAP-α Tenascin Metalloproteinases	Epithelial tumors Glioblastoma multiforme Epithelial tumors

Applications of monoclonal antibodies for cancer therapy





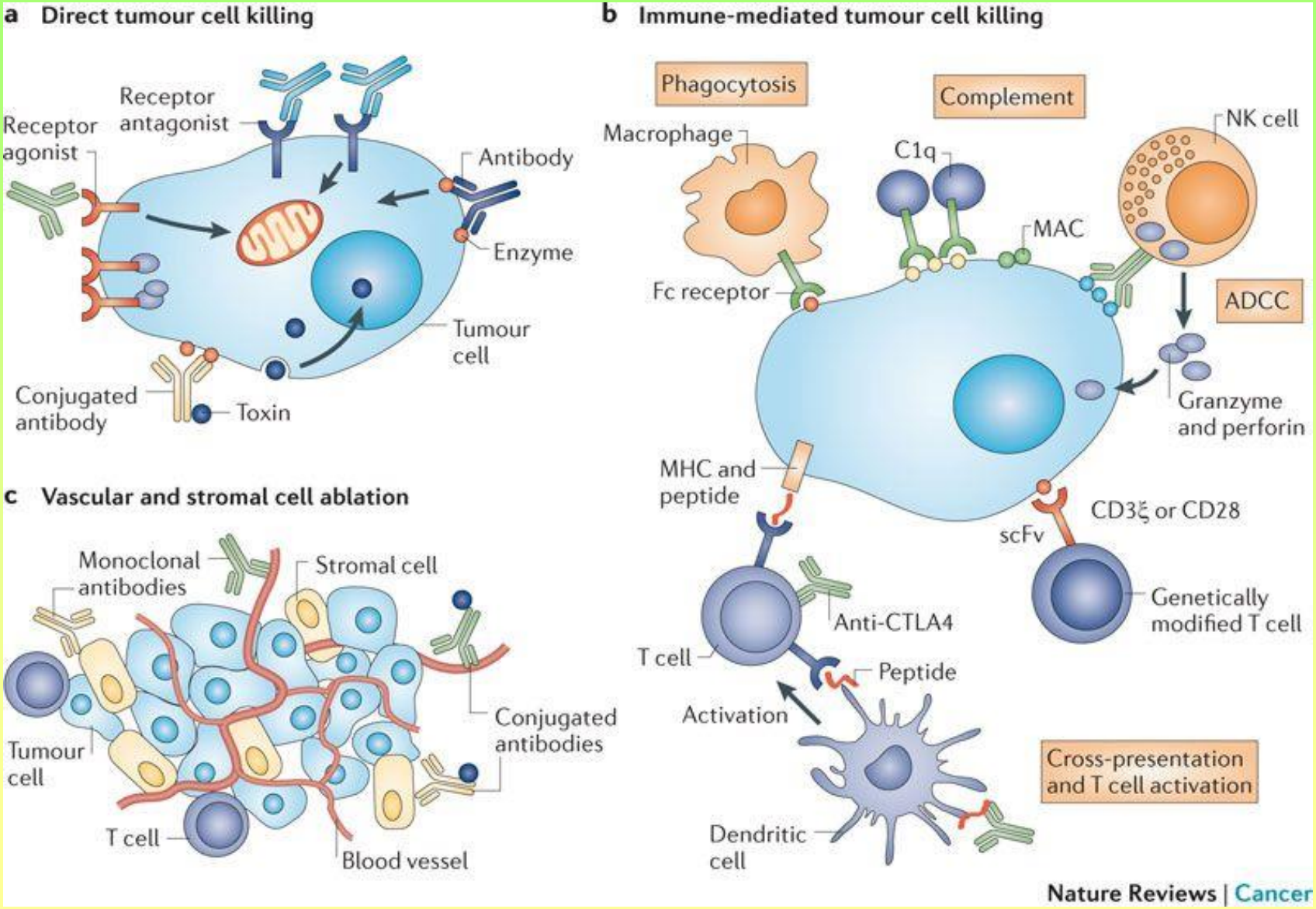
The mechanisms of action of Rituximab (anti-CD20): (1) Antibody-dependent cell-mediated cytotoxicity (ADCC). (2) Complement-dependent cytotoxicity (CDC). (3) Direct effects of binding (induction of apoptosis and sensitization to other chemotherapeutic agents). (4) Antibody-dependent phagocytosis (ADP).

Selected Clinical Trials of Unconjugated Monoclonal Antibodies in Lymphoma Therapy

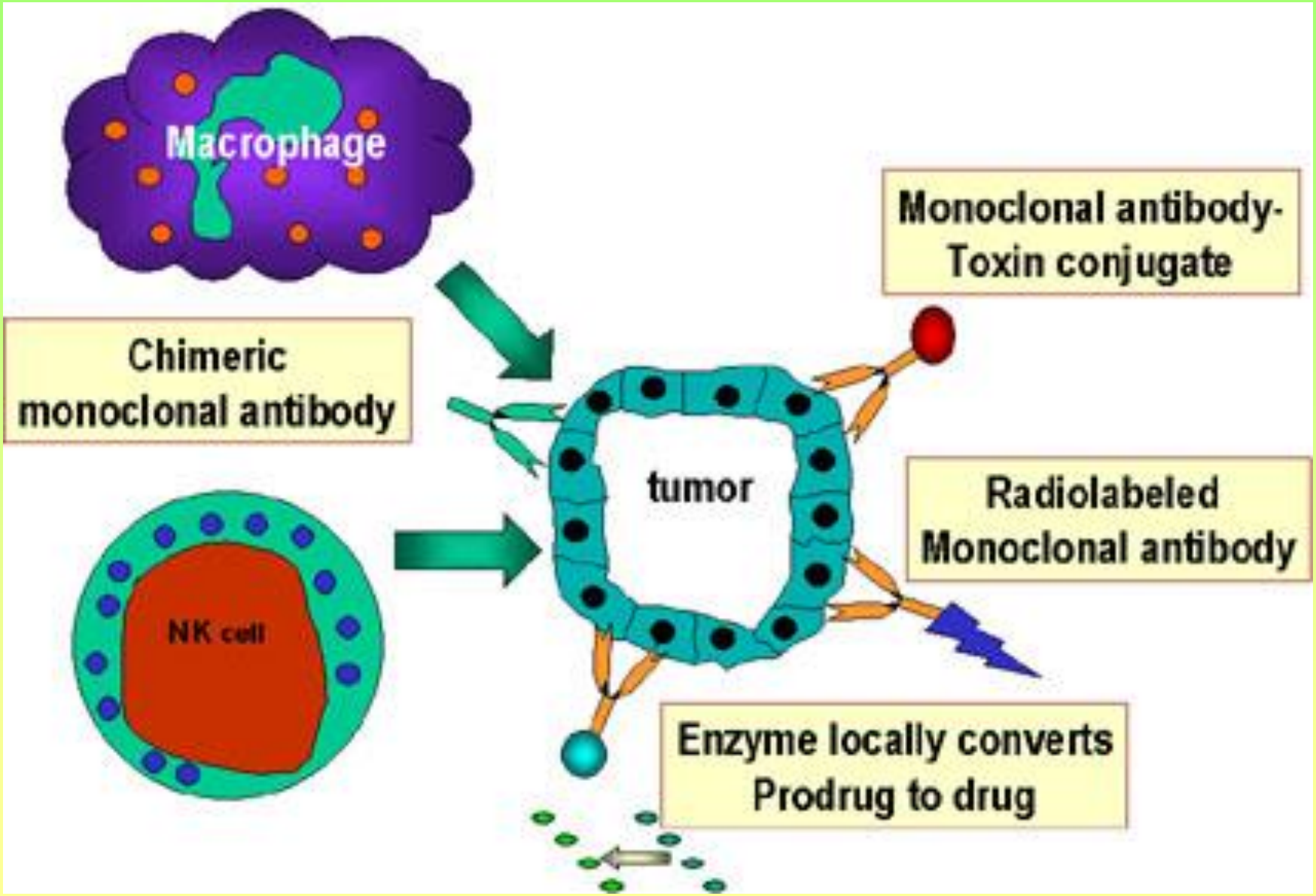
Antigen	Monoclonal Antibodies	Disease	No. of Pts	Dose	Response	Problems/Observations
Ig idiotype	Custom anti-idiotype (murine)	Relapsed B-Cell NHL	34	400-11,500 mg total qod x 2-3 wks alone, with Chl or IFN	PR, 50%, CR, 18%	Minor infusional toxicity Serum idiotype Modulation Id Id negative escape Custom MAb each pt
CD20	Rituximab (chimeric)	Relapsed low-grade B-cell NHL	204	375 mg/m² 1 x each wk x 4	PR + CR 50%	Minor infusional toxicity Fever
CD52	CAMPATH 1H (humanized)	CLL, no prior chemo	9	30 mg, tiw x 18 wk	5 PR, 3 CR	Moderate infusional toxicity Immunosuppression
		CLL, prior chemo	29	30 mg, tiw x 12 wk	11 PR,1 CR	
		T-PLL	15	30 mg, tiw x 12 wk	2 PR, 9 CR	
CD4 (chimeric)	CMT412	CTCL	15	50-200 mg single or 10-80 mg x biw x 6	14 improved transiently	Minimal toxicity
CD25	Anti-Tac (murine)	HTLV-1 induced adult T-cell leukemia	19	100-220 mg over 5-16 days	4 PR, 2 CR	Minimal toxicity
CD5	L17F12 T-101 (murine)	CTCL B-CLL	35 25	1-500 mg multiple schedules	Transient responses CTCL > CLL	Mild infusional toxicity Modulation HAMA
CD19	CLB-CD19 (murine) ± IL-2	B-cell NHL	6 7	15-250 mg x 4 days various twice weekly	1 PR 1 PR	Minimal toxicity Modulation IL-2 toxicity
HLA-DR	Lym-1 (murine)	NHL	10	58-465 mg x 4	3 minor	Minor infusional toxicity

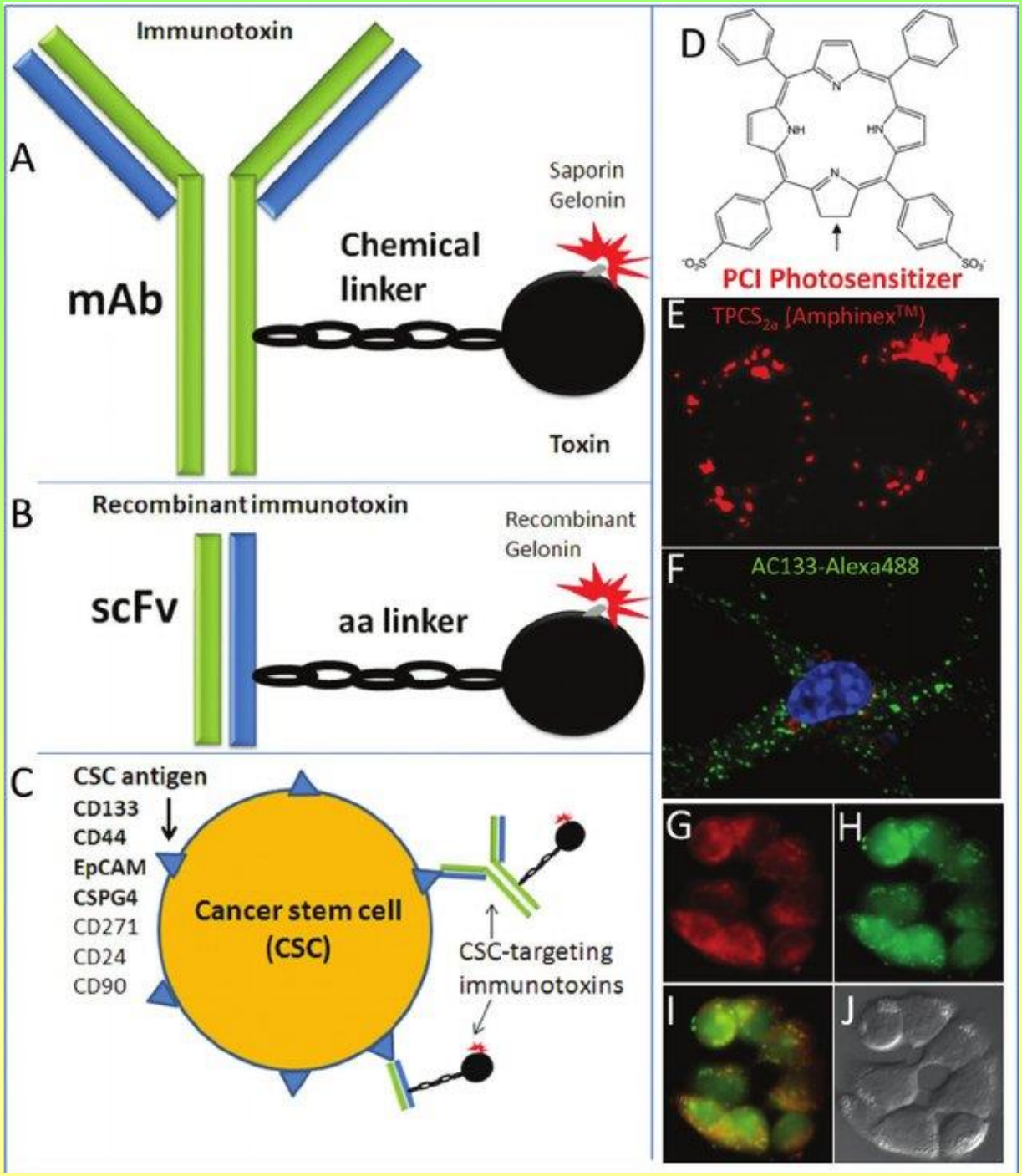
biw = 2x/week; Chl = chlorambucil; CR = complete response; HAMA = human anti-mouse antibody; Id = idiotype; IFN = Interferon; Ig = immunoglobulin; MAb = monoclonal antibody; NHL = non-Hodgkin's Lymphoma; PR = partial response; tiw = 3x/week.

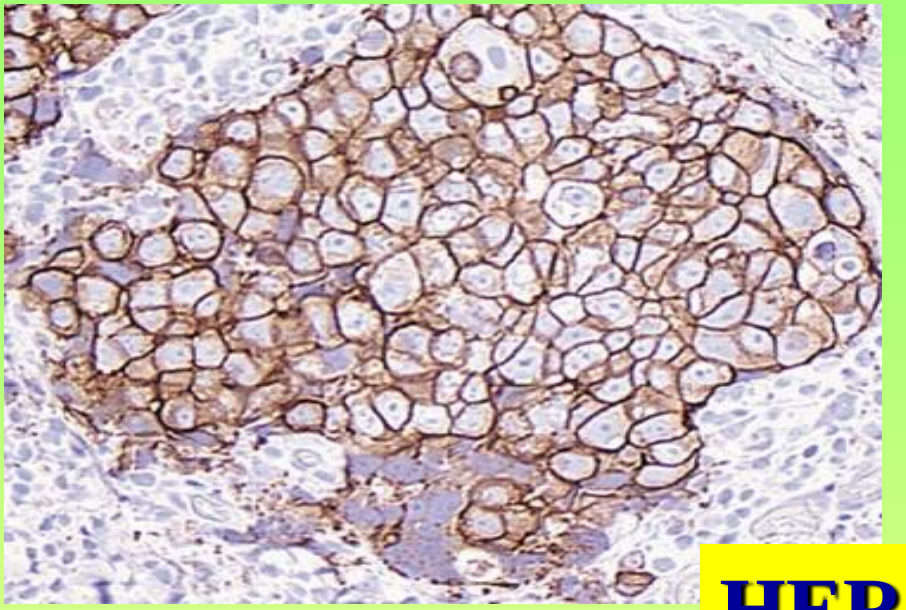
Mechanism of action of monoclonal antibodies for therapy of solid malignant tumors



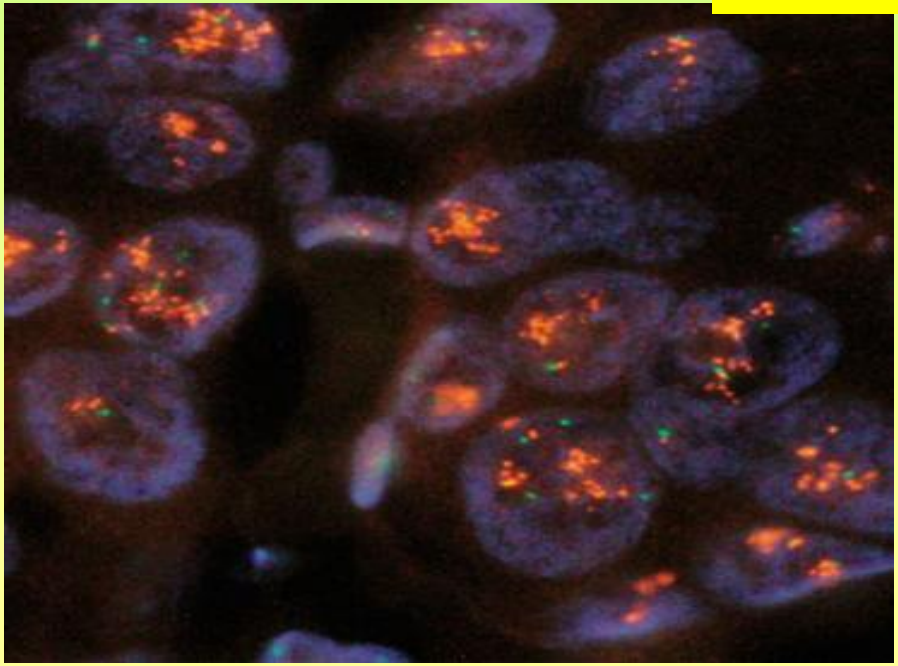
Immunotoxin therapy

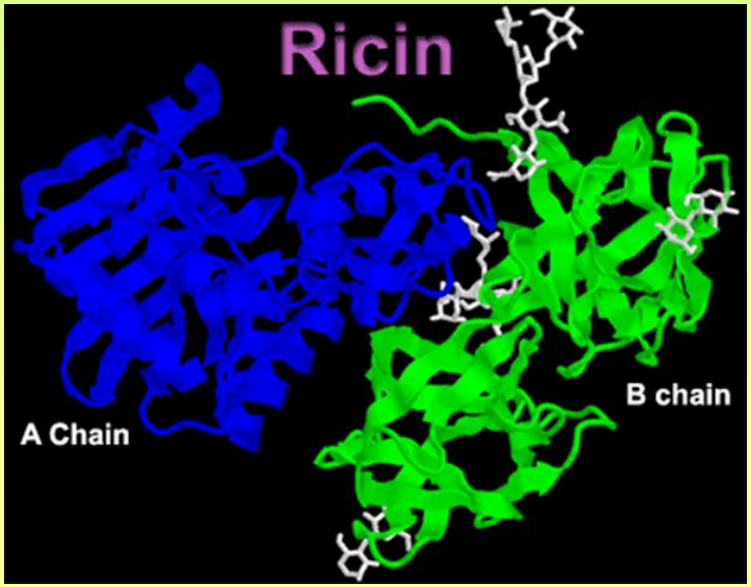



A
Immunotoxin
mAb
Chemical linker
Saporin
Gelonin
Toxin
B
Recombinant immunotoxin
scFv
aa linker
Recombinant Gelonin
C
CSC antigen
CD133
CD44
EpCAM
CSPG4
CD271
CD24
CD90
Cancer stem cell (CSC)
CSC-targeting immunotoxins
D
PCI Photosensitizer
E
TPCS2a (Amphinex™)
F
AC133-Alexa488
G
H
I
J

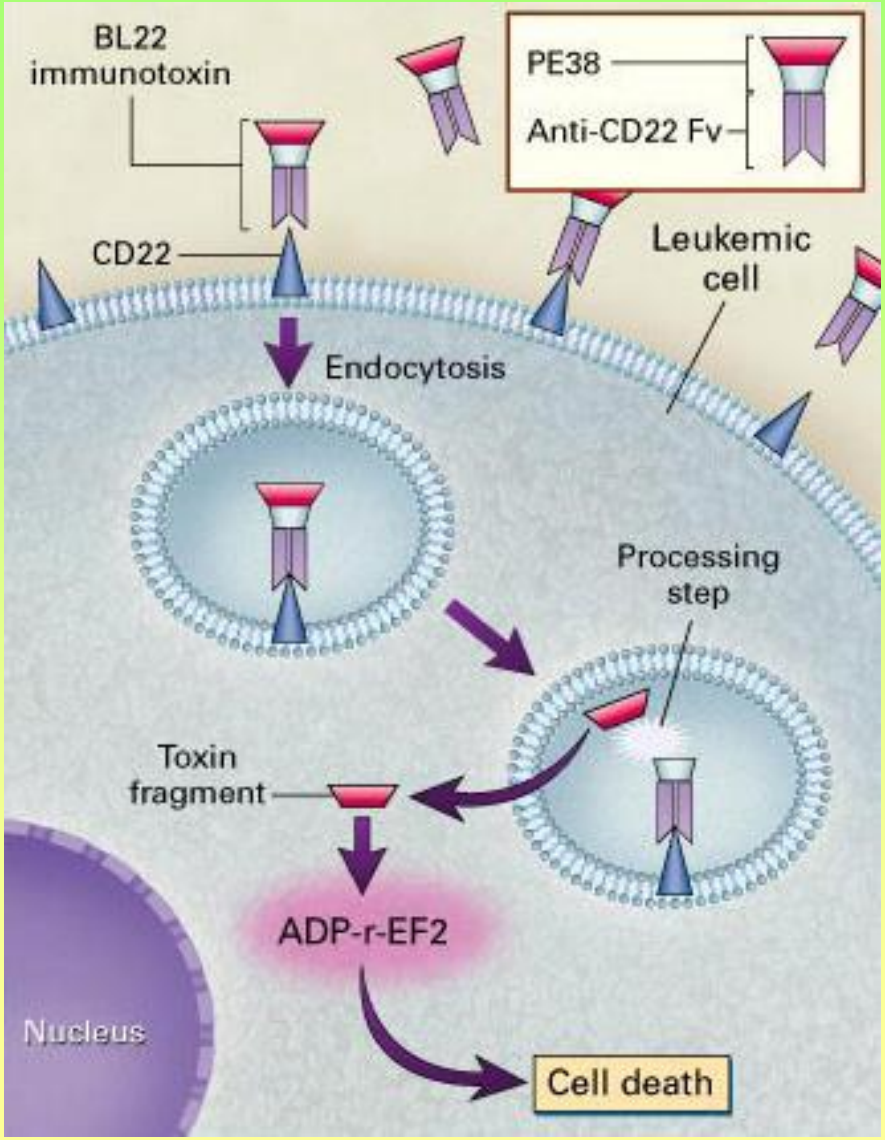


HER-2/neu

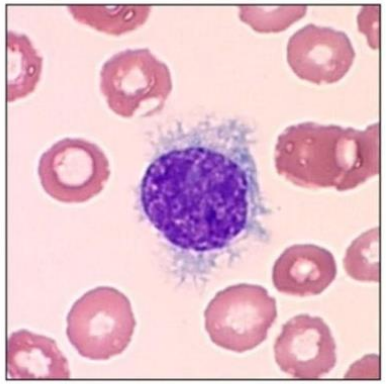




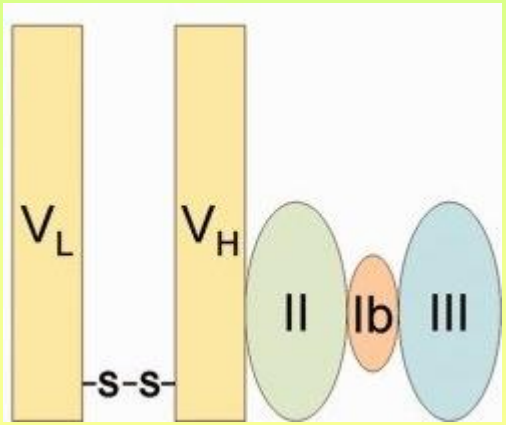
Immunotoxin therapy of „Hairy Cell" leukaemia by BL22



- Rare B-cell leukemia
- Characterized by very high CD22 expression[a]
- Often presents with pancytopenia and splenomegaly[b]
- Identifiable on peripheral blood smear due to characteristic appearance

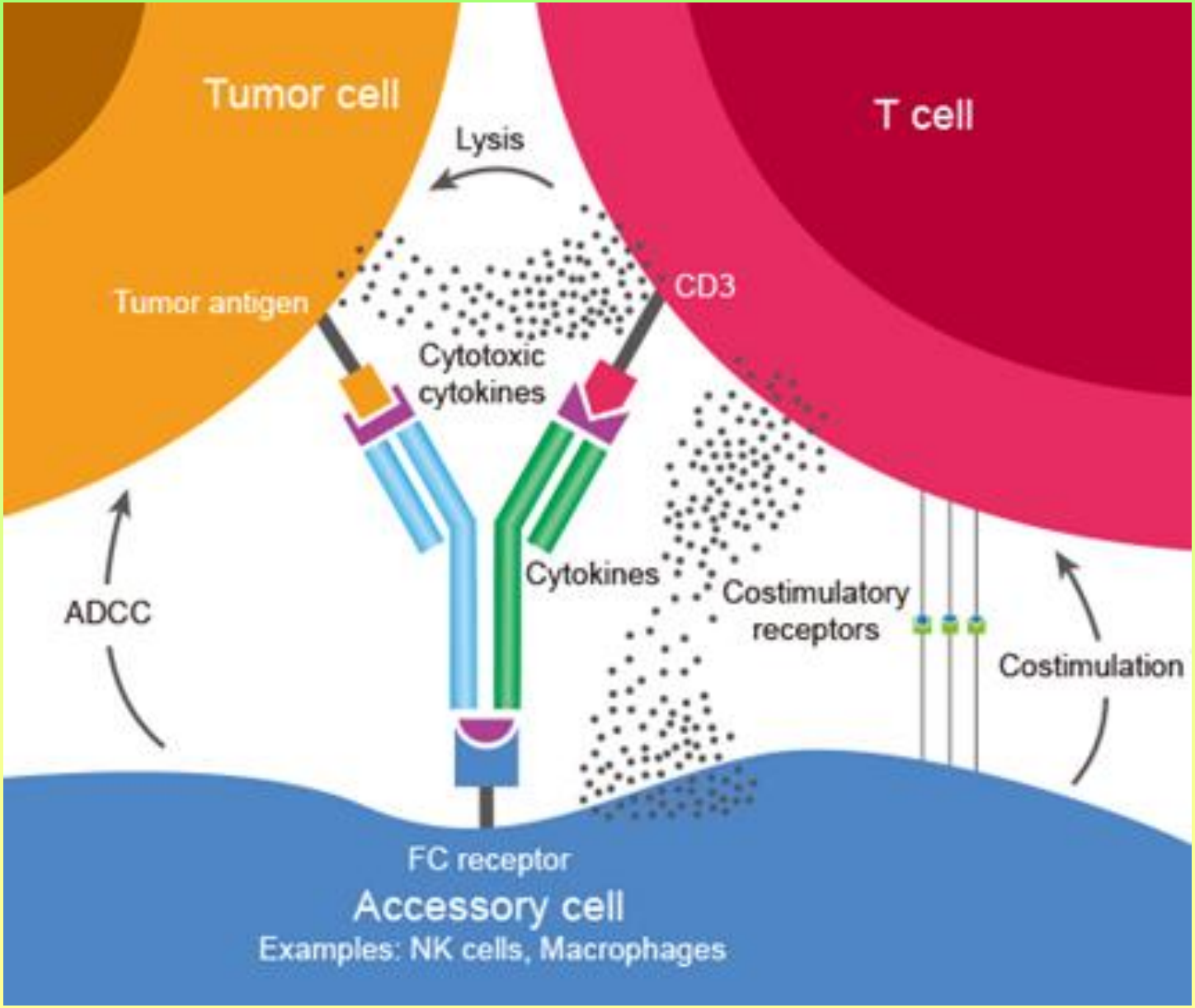


Hair-like projections of cytoplasmic membrane characteristic of hairy cell leukemia[c]

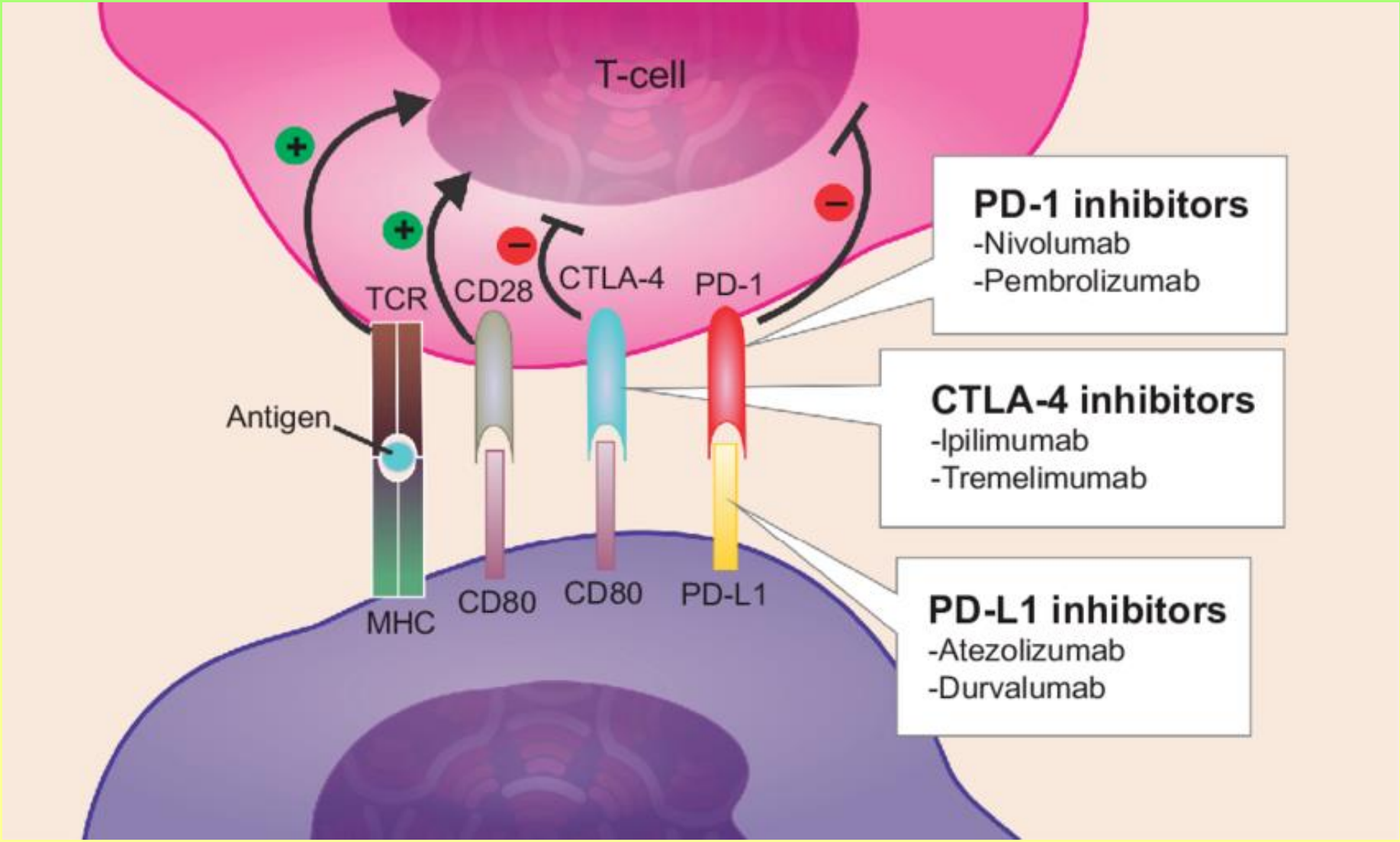


Pseudomonas exotoxin (**pe38**) conjugate to Ig variable H and L chains

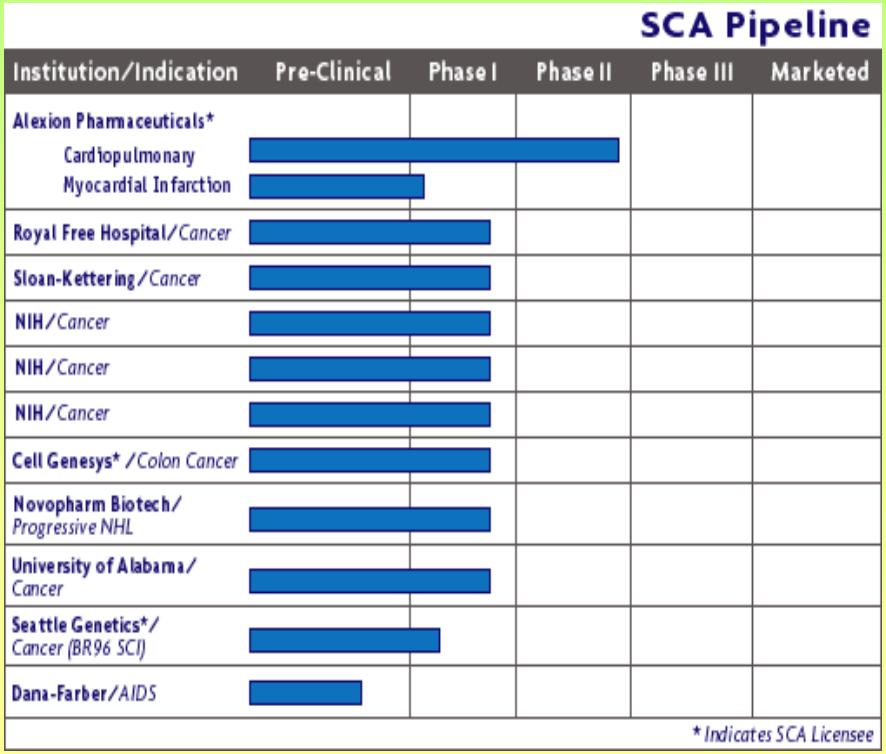
Bispecific monoclonal antibody therapy



Immune checkpoint inhibitors in cancer therapy



The first monoclonal antibodies for therapeutic use



MAB	Antibody Type	Approved Indication(s)	Year Approved
1. Orthodone OKT3 (Muromonab -- CD3; Johnson & Johnson; OrthoBiotech)	Murine	Treatment of acute kidney, heart, and liver transplant rejection	1986
2. ReoPro (Abciximab -- Centocor)	Chimeric (Hu-mu)	Adjunct to coronary intervention (angioplasty, stent, atherectomy) for prevention of coronary thrombosis	1994
3. Rituxan (Rituximab -- IDEC; Genentech)	Chimeric (Hu-mu)	Non-Hodgkin's B cell lymphoma (low-grade or follicular)	1997
4. Zenapax (Dadizumab -- Protein Design Labs; Hoffman-La Roche)	Humanized (Hu-mu)	Prevention of acute kidney transplant rejection	1997
5. Simulect (Basiliximab -- Novartis)	Chimeric (Hu-mu)	Prevention of acute kidney transplant rejection	1998
6. Synagis (Palivizumab -- MedImmune)	Humanized (Hu-mu)	Prevention of respiratory syncytial virus infection in pediatric patients	1998
7. Remicade (Infliximab -- Centocor)	Chimeric (Hu-mu)	Crohn's disease	1998
8. Herceptin (Trastuzumab -- Genentech)	Humanized (Hu-mu)	Metastatic breast cancer	1998
9. Mylotarg (American Home Products)	Humanized (toxin-linked)	Acute myelogenous leukemia	2000
10. Campath (Millennium Pharmaceuticals)	Humanized	Chronic lymphocytic leukemia	2001
11. Zevalin (IDEC Pharmaceuticals)	Chimeric (Hu-Mu; radionuclide-linked)	Non-Hodgkins lymphoma	2002

Therapeutic monoclonal antibodies in the US between 1986 and 2001

Table 3. Therapeutic monoclonal antibodies approved by the US FDA

Generic name	Trade name	Sponsor company	Type	Approval date
Muromonab-CD3	Orthoclone	Ortho Biotech	Murine	1986
Abciximab	ReoPro	Centocor	Chimeric	1994
Rituximab	Rituxan	Genentech	Chimeric	1997
Daclizumab	Zenapax	Hoffman-La Roche	Humanized	1997
Basiliximab	Simulect	Novartis	Chimeric	1998
Palivizumab	Synagis	MedImmune	Humanized	1998
Infliximab	Remicade	Centocor	Chimeric	1998
Trastuzumab	Herceptin	Genentech	Humanized	1998
Gemtuzumab ozogamicin	Mylotarg	Wyeth-Ayerst	Humanized	2000
Alemtuzumab	Campath	Millennium/ILEX	Humanized	2001

2004: more than 400 under clinical trials in the US (including biosimilars)

2013: more than 2000 under clinical trials in US and EU (including biosimilars)

2017-2020: more than 70 new products introduced in the market

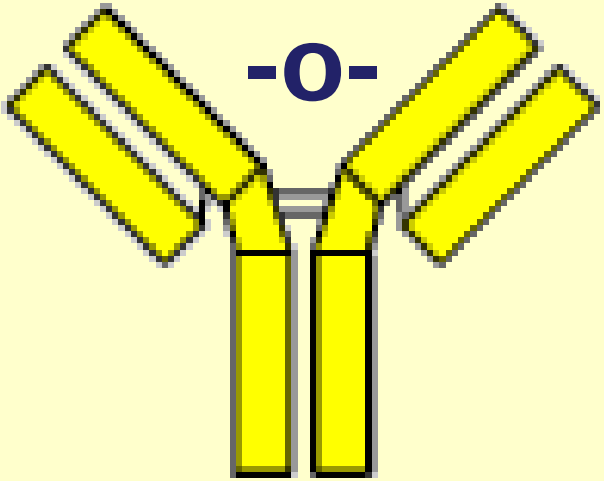
Main category	Type	Application	Mechanism/Target	Mode
Anti-inflammatory	**infliximab**	heumatoid arthritis Crohn's disease Ulcerative Colitis	inhibits TNF-α	chimeric
	adalimumab	rheumatoid arthritis Crohn's disease Ulcerative Colitis	inhibits TNF-α	human
	basiliximab	Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	chimeric
	daclizumab	Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	humanized
	omalizumab	moderate-to-severe allergic asthma	inhibits human immunoglobulin E (IgE)	humanized
Anti-cancer	**gemtuzumab**	relapsed acute myeloid leukemia	targets myeloid cell surface antigen CD33 on leukemia cells	humanized
	alemtuzumab	B cell leukemia	targets an antigen CD52 on T- and B-lymphocytes	humanized
	rituximab	non-Hodgkin's lymphoma	targets phosphoprotein CD20 on B lymphocytes	chimeric
	trastuzumab	breast cancer with HER2/neu overexpression	targets the HER2/neu (erbB2) receptor	humanized
	nimotuzumab	Approved in squamous cell carcinomas, Glioma Clinical trials for other indications underway	EGFR inhibitor	humanized
	cetuximab	Approved in squamous cell carcinomas, colorectal carcinoma	EGFR inhibitor	chimeric
	bevacizumab	Anti-angiogenic cancer therapy	inhibits VEGF	humanized
Other	**palivizumab**	RSV infections in children	inhibits an RSV fusion (F) protein	humanized
	abciximab	Prevent coagulation in coronary angioplasty	inhibits the receptor GpIIb/IIIa on platelets	chimeric

Nomenclature of therapeutic mononclonal antibodies

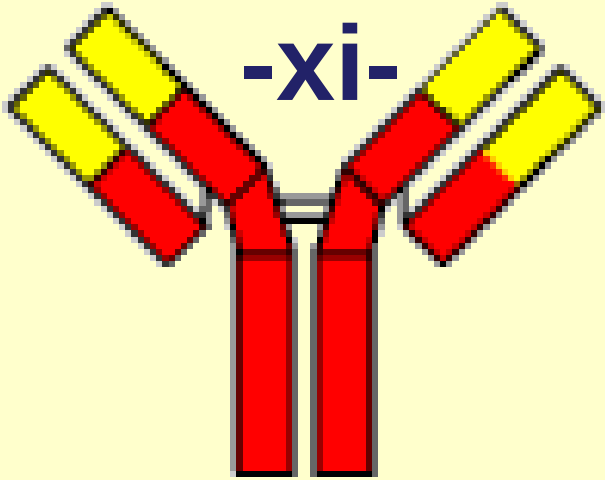
Prefix (variable) – **Target** – **Origin** – **mab**

(E.g. *anti*-CD20 ***Ri tu xi mab***)

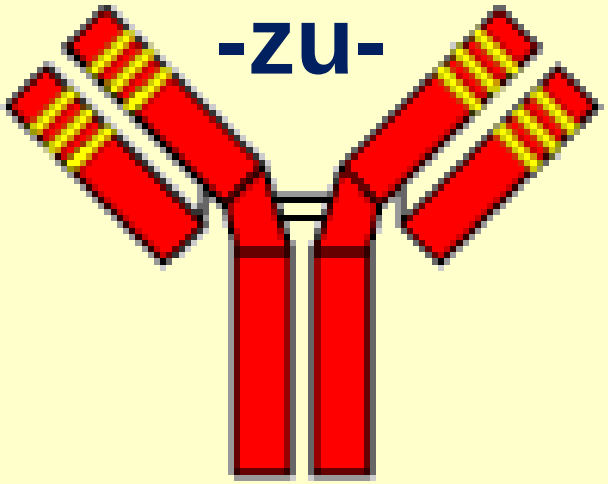
TARGET		ORIGIN	
b(a)	bacterium	-a-	rat
c(i)	circulatory system	-e-	hamster
f(u)	fungus	-i-	primat
k(i)	interleukin	-o-	mouse
l(i)	immune system	-u-	human
n(e)	nervous system	-xi-	chimeric
s(o)	bone	-zu-	humanized
tox(a)	toxin	-xizu-	chimeric/humanized hybrid
t(u)	tumor	-axo-	rat/mouse hybrid
v(i)	virus		



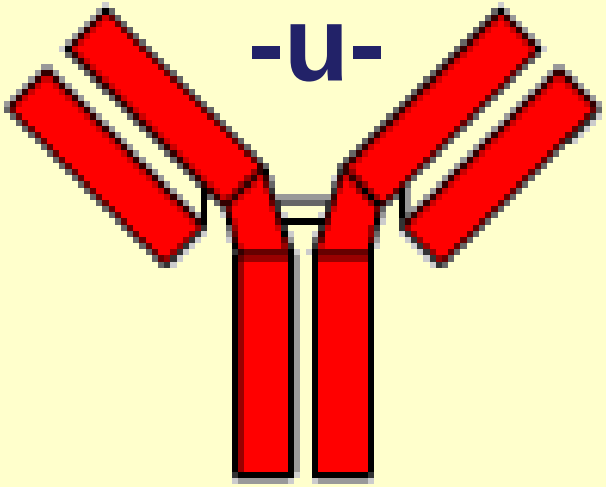
Murine



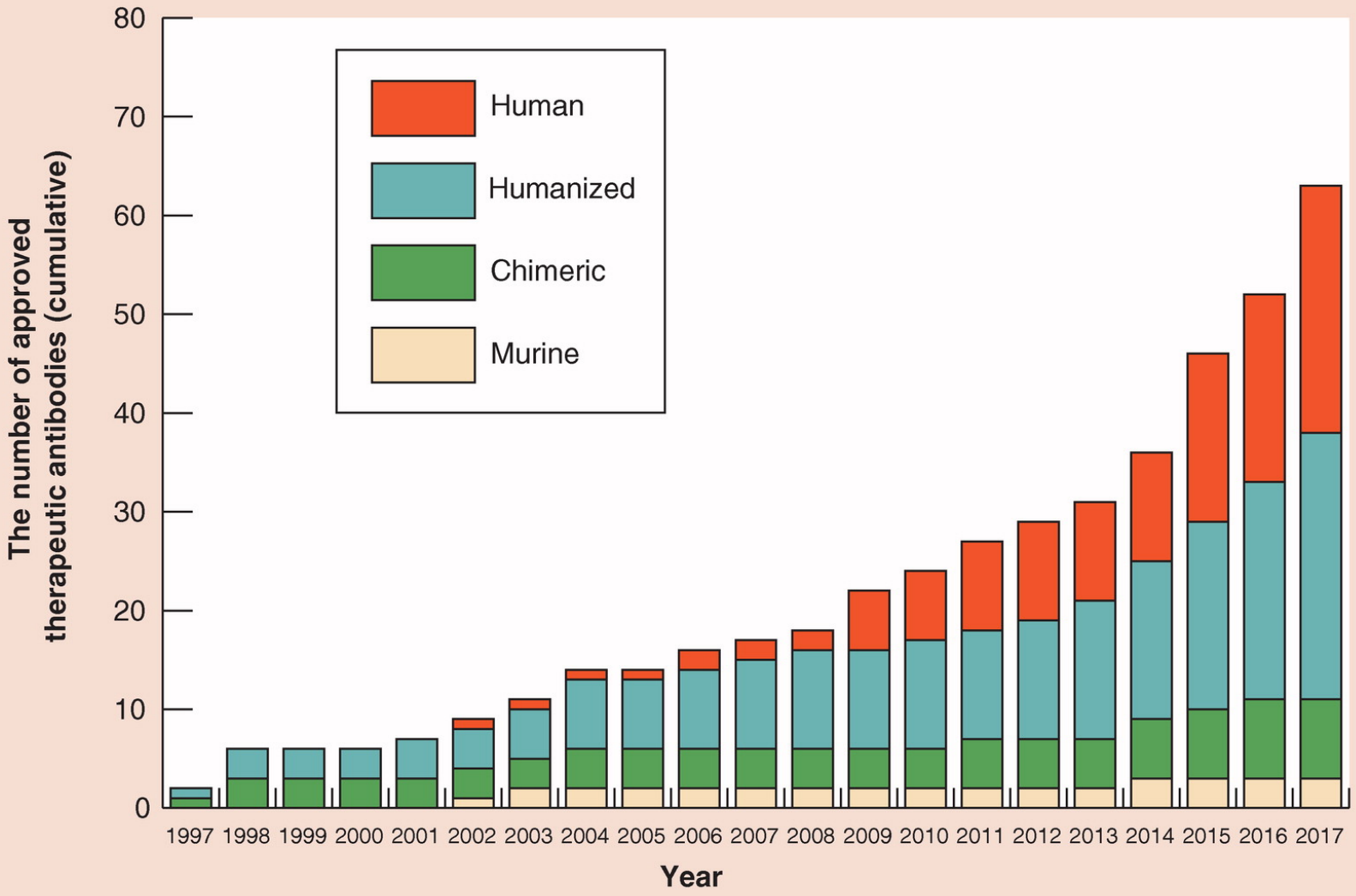
Chimaeric



Humanised



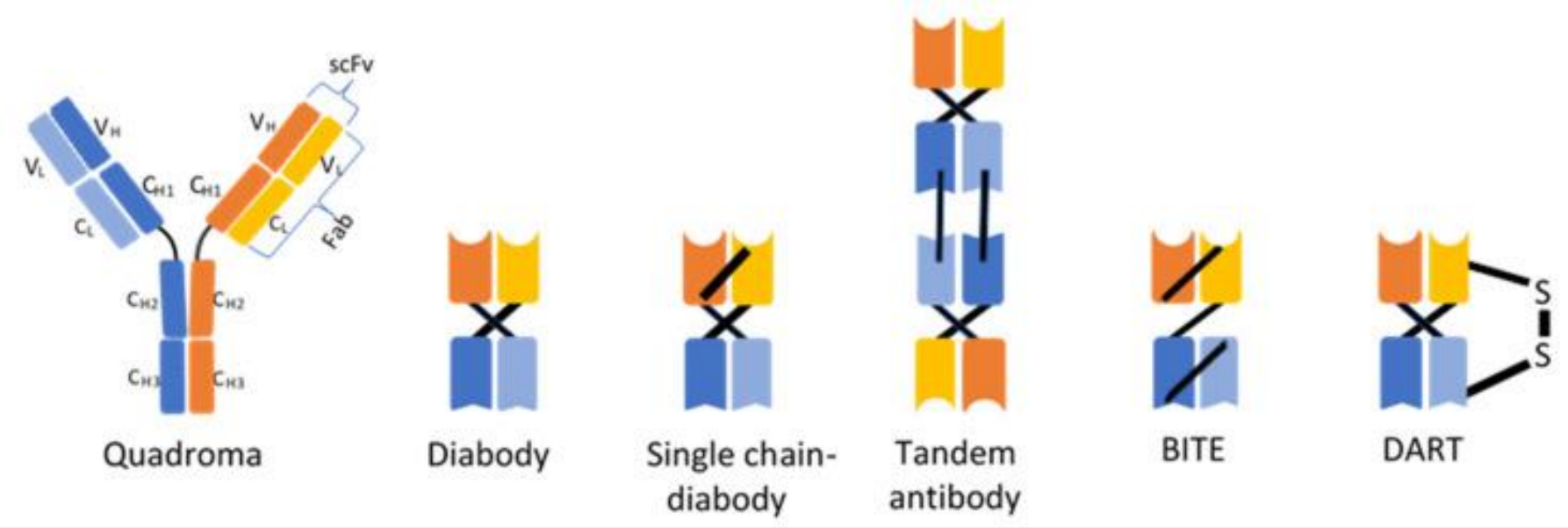
Human


Human
Humanized
Chimeric
Murine
The number of approved therapeutic antibodies (cumulative)
0
10
20
30
40
50
60
70
80
1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017
Year

Over 500 mabs used for human therapeutic application in 2020

More than 2000 are under clinical trial at the moment

Selected recombinant antibodies developed for anti-cancer therapies

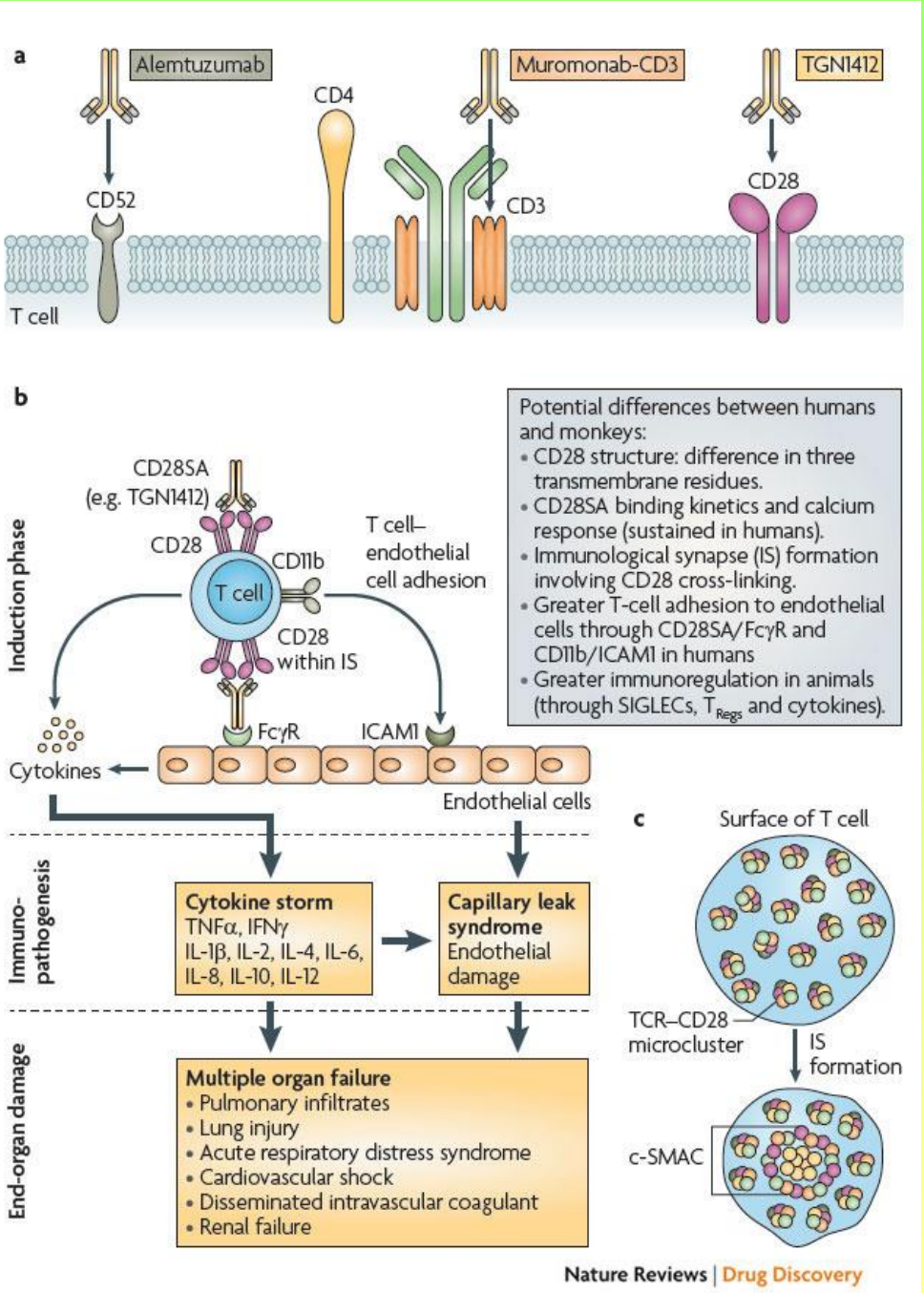


BITE—bispecific T-cell engager, DART—Dual affinity retargeting.

Monika A. Papież et al: Biological Therapies in the Treatment of Cancer—Update and New Directions. Int J Mol Sci. 2021 Nov; 22(21): 11694.

Risk of mab treatments

- Allergic reactions
- Intolerance
- Declined and blocked activity
- Banal infections
- Tuberculosis
- Sever, non-specific immunological side effects



a | Surface receptors on T cells can cause a **cytokine storm** when activated by therapeutic mAbs (Alemtuzumab, Muromonab-CD3 and TGN1412). **b** | TGN1412 can directly cause some cytokine release, as CD28 is expressed on a variety of cells in the normal immune system. Cross-linking of human CD28 may contribute to the formation of an activated immunological synapse (IS) on the surface of T cells, and binding of CD28SA to Fcγ receptors (FcγRs) on endothelial cells and other leukocytes could cause further cytokine release. Activation of CD28 may also cause **upregulation of adhesion molecules** such as CD11b on the surface of T cells or other cells of the innate immune system, which can then bind to intracellular adhesion molecule 1 (ICAM1) on endothelial cells. T cell–endothelial complexes have the capacity to cause **amplified cytokine production and local endothelial damage**. Hence, the cytokine storm and neutrophil infiltration could mediate the **capillary leak syndrome with resultant multiple organ failure**. **c** | The IS forms in a dynamic process on the T-cell plasma membrane, in which the five components of the TCR–CD28 microcluster aggregate to form a central supramolecular activation cluster (c-SMAC). The latter consists of a core of TCR and CD3 molecules, surrounded by a ring of CD28 molecules with associated protein kinase Cθ, which causes **sustained T-cell activation**.