Therapeutic monoclonal antibodies

ANTIBODIES

•produced by the B cells (= B lymfocytes, a subgroup of agranulocytes) of the immune system.

•like weapons of our defense system and can be described as “ homing devices ” that target antigens and destroy them.

•immunoglobulins of 5 classes:

•immunoglobulin G and D (IgG and IgD, ~ 75%)

•immunoglobulin A (IgA ~ 15%)

• immunoglobulin M (IgM ~ 15%)

•immunoglobulin E (IgE < 1%).

They differ from each other in size, charge, carbohydrate content, and amino acid composition. Within each class, there are subclasses that show slight differences in structure and function from other members of the class.

Antibody structure

•normally depicted as a capital letter Y configuration

•IgG is the most predominant antibody: a tetrameric molecule consisting of two identical heavy (H) polypeptide chains of about 440 amino acids and two identical light (L) polypeptide chains of about 220 amino acids

• four chains are held together by disulfide bonds and noncovalent interactions

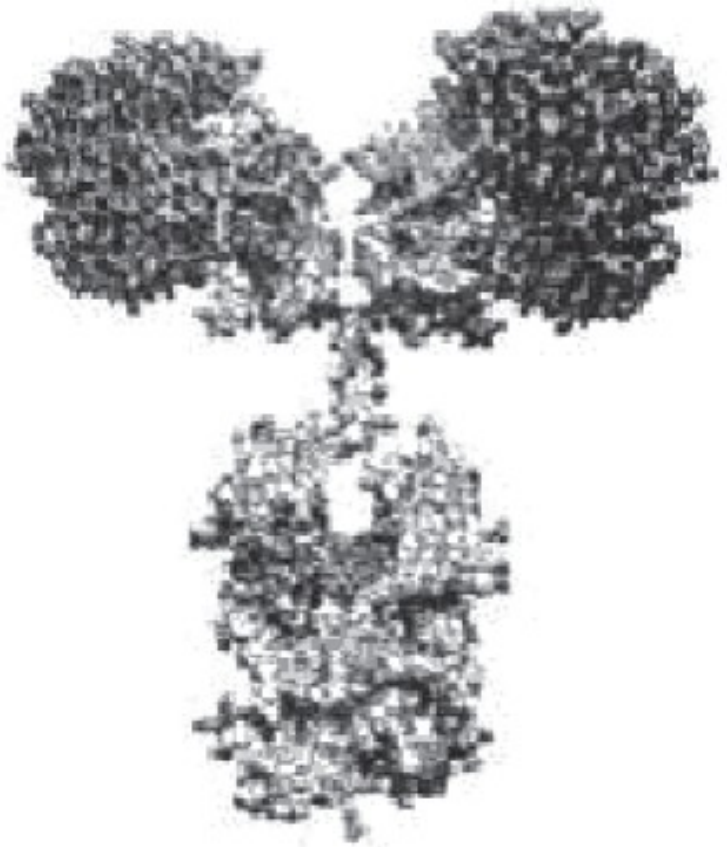
•within the light and heavy chains are domains, which consist of about 110 amino acids of similar polypeptide sequence: constant domains C_H1, C_H2, and C_H3 of the heavy chain

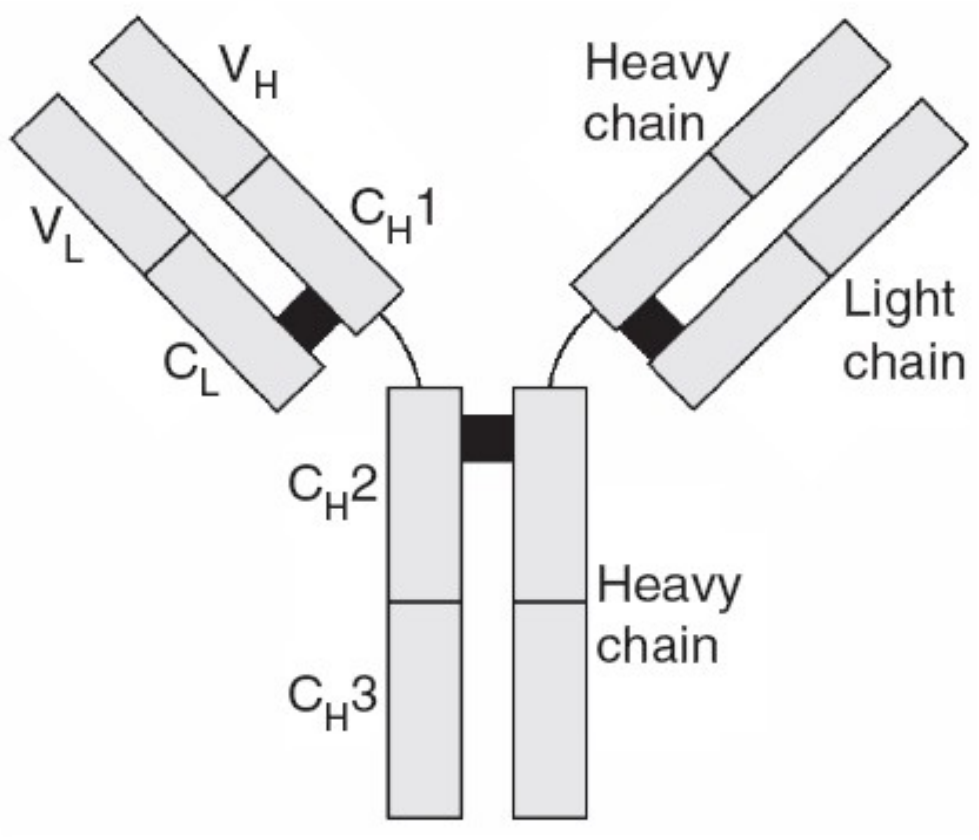
C_L domain of the light chain

•where the sequence is variable, variable domains, one each on the heavy and light chain: V_H and V_L

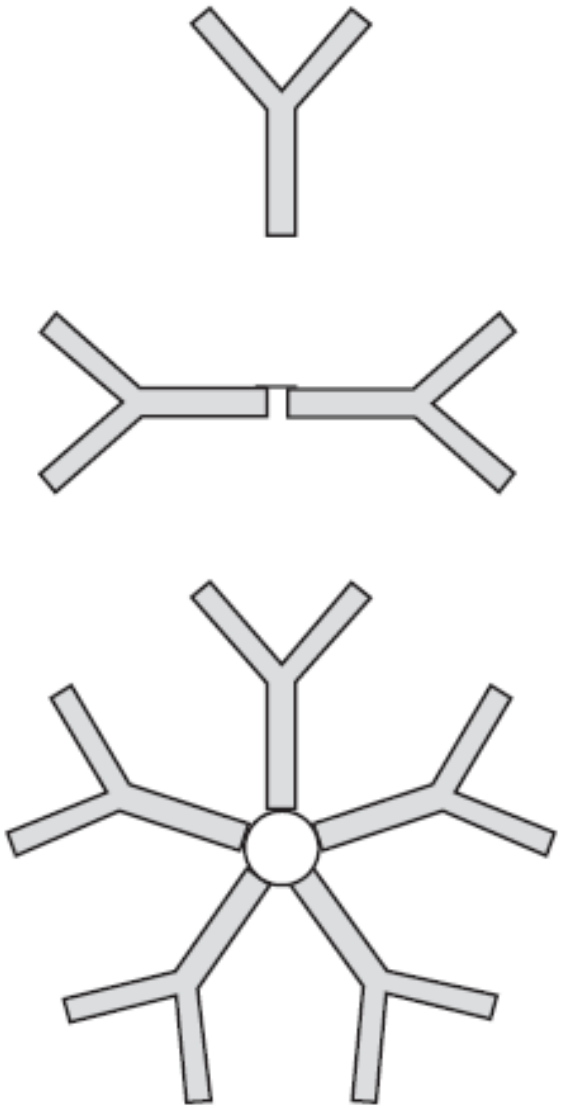
•the variability is confined to particular regions of the variable domain, called the complementarity - determining regions. These regions have the appropriate 3D structure to bind to antigens.

Antibody structure





Typical quartenary structure of immunoglobulins



IgG, IgD, IgE
(monomer)

IgA
(dimer)

IgM
(pentamer)

Antibody structure

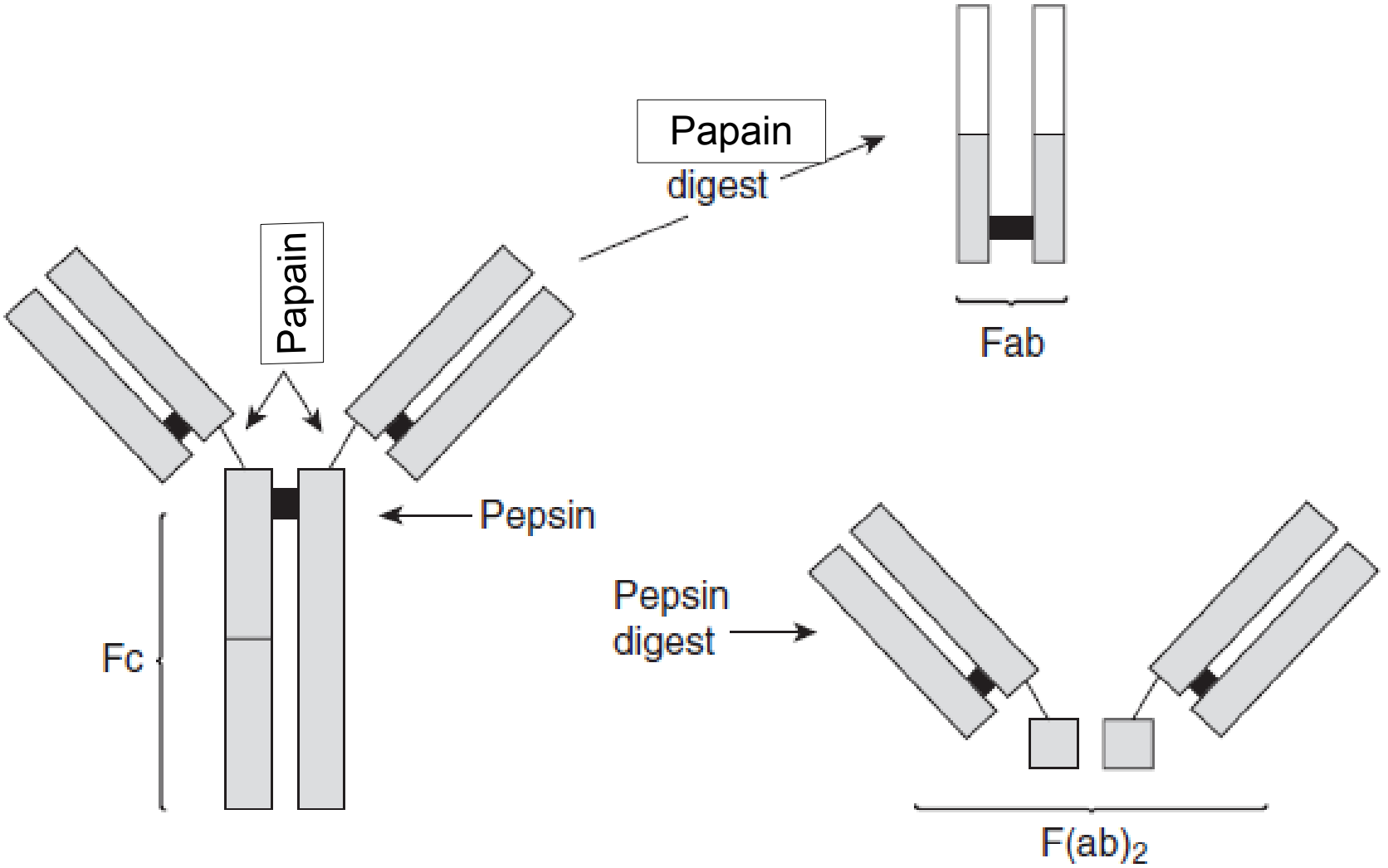
An antibody can be cleaved by enzymes such as papain and pepsin into different fragments.These different fragments are the following:

• Variable Fragment (Fv): The tips of the two Y arms vary greatly from one antibody to another. They are the regions that bind to epitopes of antigens and bring them to the natural killer cells and macrophages for destruction.

• Antigen - Binding Fragments (Fab), Fab′ , and F $(ab')_2$: Various parts that contain the variable fragment.

• Constant Fragment (Fc): This is the stem of the letter Y . It is the part that is identical for all antibodies of the same class; for example, all IgGs have the same Fc. The Fc fragment is the part that links the antibody to other receptors and triggers immune response and antigen destruction.

Different fragments of the antibody molecule



Monoclonal antibodies for human use as defined by the Pharmacopoeia (EP 7.5)
Anticorpora monoclonalia ad usum humanum

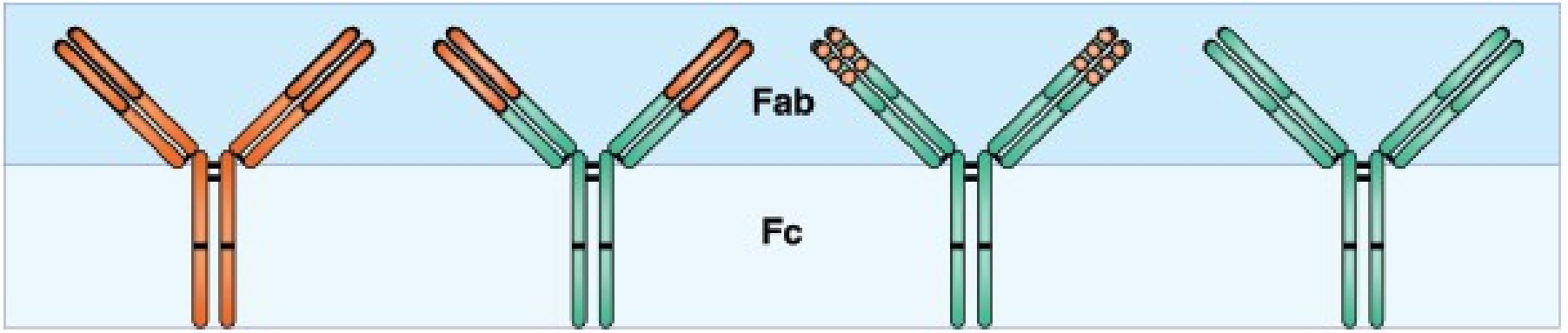
Monoclonal antibodies for human use are preparations of an immunoglobulin or a fragment of an immunoglobulin, for example, $F(ab')_2$, with defined specificity, produced by a single clone of cells. They may be conjugated to other substances, including for radiolabelling. They can be obtained from immortalised B lymphocytes that are cloned and expanded as continuous cell lines or from rDNA-engineered cell lines.

Currently available rDNA-engineered antibodies include the following antibodies.
Chimeric monoclonal antibodies: the variable heavy- and light-chain domains of a human antibody are replaced by those of a non-human species that possess the desired antigen specificity.
Humanised monoclonal antibodies: the 3 short hypervariable sequences (the complementarity-determining regions) of non-human variable domains for each chain are engineered into the variable domain framework of a human antibody; other sequence changes may be made to improve antigen binding.
Recombinant human monoclonal antibodies: the variable heavy- and light-chain domains of a human antibody are combined with the constant region of a human antibody.

⇓

The EP does not suppose fully animal mabs in current use in humans.

Examples of mabs in therapeutical use woldwide



Prefix	Target			Antibody Source		Suffix	Some Examples
Variable	Non-tumor Target	Viral	-vir-	-u-	Human	-mab	Pali-vi-zu-mab (humanized antiviral Mab)
		Bacterial	-bac-				
		Immune	-lim-	-o-	Murine		Ada-lim-u-mab (human Mab against immune disease target)
		Infectious lesions	-les-				
		Antifungal	-fung-	-a-	Rat		E-fung-u-mab (human antifungal Mab)
		Cardiovascular	-ci(r)-				
		Neurologic	-ne(r)-	-e-	Hamster		Bapi-neu-zu-mab (humanized Mab against neurobiology target)
		Interleukins	-kin-				
		Musculoskeletal	-mul-	-i-	Primate		Uste-kin-u-mab (human anticytokine Mab)
		Bone	-os-				
		Toxin as target	-toxa-	-xi-	Chimeric		
	Tumor target	Colon	-col-				Den-os-u-mab (human antibone target Mab
		Melanoma	-mel-	-zu-	Humanized		
		Mammary	-mar-				Ab-ci-xi-mab (chimeric Mab against CV target)
		Testis	-got-	-axo-	Rat/murine hybrid		
		Ovary	-gov-				Ore-gov-o-mab (murine Mab for ovarian cancer)
		Prostate	-pr(o)-	-xizu-	Chimeric + humanized		
		Miscellaneous	-tu(m)-				Adeca-tum-u-mab (human antibody against miscellaneous tumor target)

Examples of particular therapeutical
monoclonal antibodies

Antineoplastics

Growth factors inhibitors

bevacizumab

Avastin®

•humanized: Immunoglobulin G 1 (human-mouse monoclonal rhuMAb-VEGF gamma-chain anti-human vascular endothelial growth factor), disulfide with human-mouse monoclonal rhuMAb-VEGF light chain, dimer

•angiogenesis inhibitor

•antibody against vascular endothelial growth factor (VEGF)

•vascular endothelial growth factor (VEGF), a pro-angiogenic factor, binds to 2 receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), activates receptor tyrosin kinase (RTK) and induces angiogenesis

• VEGF and its receptors are often over-expressed in cancers, that is why angiogenesis was proposed as a target site of anti-cancer therapy by Folkman and col. in 1970th

•bevacizumab approved in USA in 2004 for treatment of metastatic colorectal cancer combined with fluorouracil; later against non small cells lung cancer (2006) and breast cancer (2008); for the same purposes approved also in EU

•its efficacy, either alone or in combnation, was demonstrated also in many other cancers including neuroendocrinous ones, which are often resistant to classical chemotherapy

•recently, falsifications of Avastin ® has been catched in U.K.

ramucirumab

syn. IMC-1121B

•humanized

•angiogenesis inhibitor

•targeted against VEGFR-2 receptor

•VEGFR-2 is selectively expressed in cancer endothelial cells, simultaneously it is in a direct contact with blood $\Rightarrow$ promising therapeutical target

•antibodies against Flk-1 isoform antagonised binding of VEGF to the receptor, signal transduction by means of VEGFR-2 and VEGF induced endothelial cells growth $\Rightarrow$ antiangiogenic, antitumor and antimetastatic activity

•clinical trials: phase 2 for breast cancer , phase 3 for non small lungs cells carcinoma combined with docetaxel, phase 2 for prostate cancer combined with mitoxantron and prednisone, phase 3 for gastric cancer etc.

trastuzumab
Herceptin ®

- humanized
- IgG1 κ anti – HER2

•family of receptors for epidermal growth factor includes 4 structurally very similar receptors: Erb/HER (EGFR; HER-1, and ERBB1), human EGFR-2 (HER-2 and

•ERBB2), HER-3, a HER-4, transmembrane glycoproteins containing a domain binding an intracellular ligand and an intracellular receptor tyrosine kinase (RTK) domain

•deregulation of Erb/HER pathway by over-expression or by constitutive activation can trigger a cancer process including angiogenesis and metastasising and brings a bad prognosis in many types of human cancers

•early studies with trastuzumab as a single agent in HER-2-positive metastatic breast cancer achieved overall responses of 11.6 and 15% for patients who had progressed after chemotherapy. As a first-line treatment for metastatic breast cancer, trastuzumab showed response rates of 26% in HER-2-positive patients and responses of 35% in patients with 3+ HER-2 overexpression by immunohistochemistry and 34% in patients positive for HER-2 gene amplification by fluorescence in situ hybridisation (FISH).

•a pivotal phase III trial of trastuzumab in combination with chemotherapeutic agents demonstrated an overall response rate of approximately 50% (versus 32%), longer duration of response (time to progression; 7.4 versus 4.6 months), **longer survival (overall survival: 25.1 versus 20.3 months)** and a 20% reduction in risk of death compared to chemotherapy alone in HER-2 overexpressing metastatic breast cancer.

cetuximab

Erbitux®

- chimeric IgG_1 antibody
- binds to the EGFR with an affinity that is approximately 5- to 10-fold higher than that of endogenous ligands
- blocks binding of endogenous EGFR ligands resulting in inhibition of the function of the receptor
- further induces the internalisation of EGFR, which can lead to down-regulation of EGFR
- also targets cytotoxic immune effector cells towards EGFR-expressing tumour cells (antibody dependent cell-mediated cytotoxicity, ADCC)
- in both *in vitro* and *in vivo* assays, cetuximab inhibits the proliferation and induces apoptosis of human tumour cells that express EGFR. *In vitro* cetuximab inhibits the production of angiogenic factors by tumour cells and blocks endothelial cell migration. *In vivo* cetuximab inhibits expression of angiogenic factors by tumour cells and causes a reduction in tumour neo-vascularisation and metastasis.
- cetuximab approved by both FDA and EMA for treatment of metastasising colorectal carcinomas expressing EGFR

panitumumab
Vectibix ®

- fully human monoclonal IgG_2 antibody against EGFR
- produced in a mammalian cell line by recombinant DNA technology
- binds to the ligand binding domain of EGFR and inhibits receptor autophosphorylation induced by all known EGFR ligands
- its binding to EGFR results in internalisation of the receptor, inhibition of cell growth, induction of apoptosis, and decreased interleukin 8 and vascular endothelial growth factor (VEGF) production
- *in vitro* assays and *in vivo* animal studies have shown that panitumumab inhibits the growth and survival of tumour cells expressing EGFR
- no anti-tumour effects of panitumumab were observed in human tumour xenografts lacking EGFR expression
- the addition of panitumumab to radiation, chemotherapy or other targeted therapeutic agents, in animal studies resulted in an increase in anti-tumour effects compared to radiation, chemotherapy or targeted therapeutic agents alone
- unwanted skin reactions occurred in 93 % of patients

Antitumour mabs, which target various receptors and signaling proteins on the cell surface

etaracizumab

Abegrin®

- syn. **vitaxin, MEDI-522**
- humanized
- against $\alpha_v\beta_3$ integrin
- integrins: a family of receptors on the cell surface, which are responsible for exchange of information between cells and an extracellular matrix, which surrounds them (ECM)
- heterodimers composed from 1 – 10 α-subunits and 1 - 8 β-subunits
- every subtype has its specificity for a different protein of ECM
- signals, which influence growth, migration ability, differentiation, invasivity and survival of cells, are generated in a cell in response to binding of ECM components
- integrins play an important role in tumour biology; useful target of anti-cancer therapy
- $\alpha_V\beta_3$ integrins are more expressed in developing vessels than in "adult" ones; they are supposed to be an important factor of angiogenesis
- vitronectin is the primary ligand, they also interact with fibronectin and thrombospondin
- relationship of $\alpha_V\beta_3$ with i.a. vascular endothelial growth factor (VEGF) was demonstrated
- administration of a murine monoclonal antibody against $\alpha_V\beta_3$ (LM609) interrupted cancer-caused angiogenesis on a chicken chorioallantoic membrane
- the ability of the substance to stop cancer vascularisation and cause its regression without a damage of normal matured vessels was verified in murine models of various cancers *in vitro*
- **etaracizumab** is a fully humanized form
- expression of $\alpha_V\beta_3$ murine ovarian cancer and simultaneously effect of etaracizumab against it were demonstrated
- clinical tests of phases 1 – 2 for treatment of various cancers (colorectal, malignant melanoma, androgen-independent prostate cancer, kidney cancer, lymphoma) and autoimmune inflammatory diseases (plaque psoriasis, rheumatoid arthritis) were finished

alemtuzumab

MabCampath ®

- humanised $IgG_1\kappa$ monoclonal antibody specific for a lymphocyte cell surface glycoprotein of $21\,000 < M_r < 28\,000$ (CD52) expressed primarily on the surface of several types of leukocytes
- produced in mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium
- alemtuzumab was generated by the insertion of six complementarity-determining regions from an IgG_{2a} rat monoclonal antibody into a human IgG_1 immunoglobulin molecule
- causes the lysis of lymphocytes by binding to CD52, a highly expressed, non-modulating antigen which is present on the surface of essentially all B and T cell lymphocytes as well as monocytes, thymocytes and macrophages
- the antibody mediates the lysis of lymphocytes via complement fixation and antibody-dependent cell mediated cytotoxicity
- the antigen has been found on a small percentage (< 5%) of granulocytes, but not on erythrocytes or platelets
- alemtuzumab does not appear to damage haematopoietic stem cells or progenitor cells
- indicated for the treatment of patients with B-cell chronic lymphocytic leukaemia (B-CLL) for whom fludarabine combination chemotherapy is not appropriate

rituximab

Mabthera ®

•chimeric mouse/human monoclonal antibody representing a glycosylated immunoglobulin with human IgG_1 constant regions and murine light-chain and heavy-chain variable region sequences

•produced by mammalian (Chinese hamster ovary) cell suspension culture and purified by affinity chromatography and ion exchange, including specific viral inactivation and removal procedures

•binds specifically to the transmembrane antigen, CD20, a non-glycosylated phosphoprotein, located on pre-B and mature B lymphocytes

•the antigen is expressed on >95 % of all B cell non-Hodgkin's lymphomas

•CD20 is found on both normal and malignant B cells, but not on haematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissue

• this antigen does not internalise upon antibody binding and is not shed from the cell surface.

•CD20 does not circulate in the plasma as a free antigen and, thus, does not compete for antibody binding

•the Fab domain of rituximab binds to the CD20 antigen on B lymphocytes and the Fc domain can recruit immune effector functions to mediate B cell lysis. Possible mechanisms of effector-mediated cell lysis include complement-dependent cytotoxicity (CDC) resulting from C1q binding, and antibody-dependent cellular cytotoxicity (ADCC) mediated by one or more of the Fcγ receptors on the surface of granulocytes, macrophages and natural killers (NK) cells.

•rituximab binding to CD 20 antigen on B lymphocytes has also been demonstrated to induce cell death via apoptosis

•indicated for the treatment of follicular lymphoma, CD20 positive diffuse large B cell non-Hodgkin's lymphoma, chronic lymphocytic leukaemia (CLL) and severe active rheumatoid arthritis

ofatumumab

Arzerra ®

- human monoclonal antibody (IgG_1) that binds specifically to a distinct epitope encompassing both the small and large extracellular loops of the CD20 molecule
- produced in a recombinant murine cell line (NS0)
- indicated for the treatment of chronic lymphocytic leukaemia (CLL) in patients who are refractory to fludarabine and alemtuzumab
- authorised under a so-called 'conditional approval' scheme: further evidence on this medicinal product is awaited by the European Medicines Agency (EMA)
- the binding of ofatumumab to the membrane-proximal epitope of the CD20 molecule induces recruitment and activation of the complement pathway at the cell surface, leading to complement-dependent cytotoxicity and resultant lysis of tumour cells
- induces appreciable lysis of cells with high expression levels of complement defence molecules
- induces cell lysis in both high and low CD20 expressing cells and in rituximab-resistant cells
- in addition, the binding of ofatumumab allows the recruitment of natural killer cells allowing the induction of cell death through antibody-dependent cell-mediated cytotoxicity

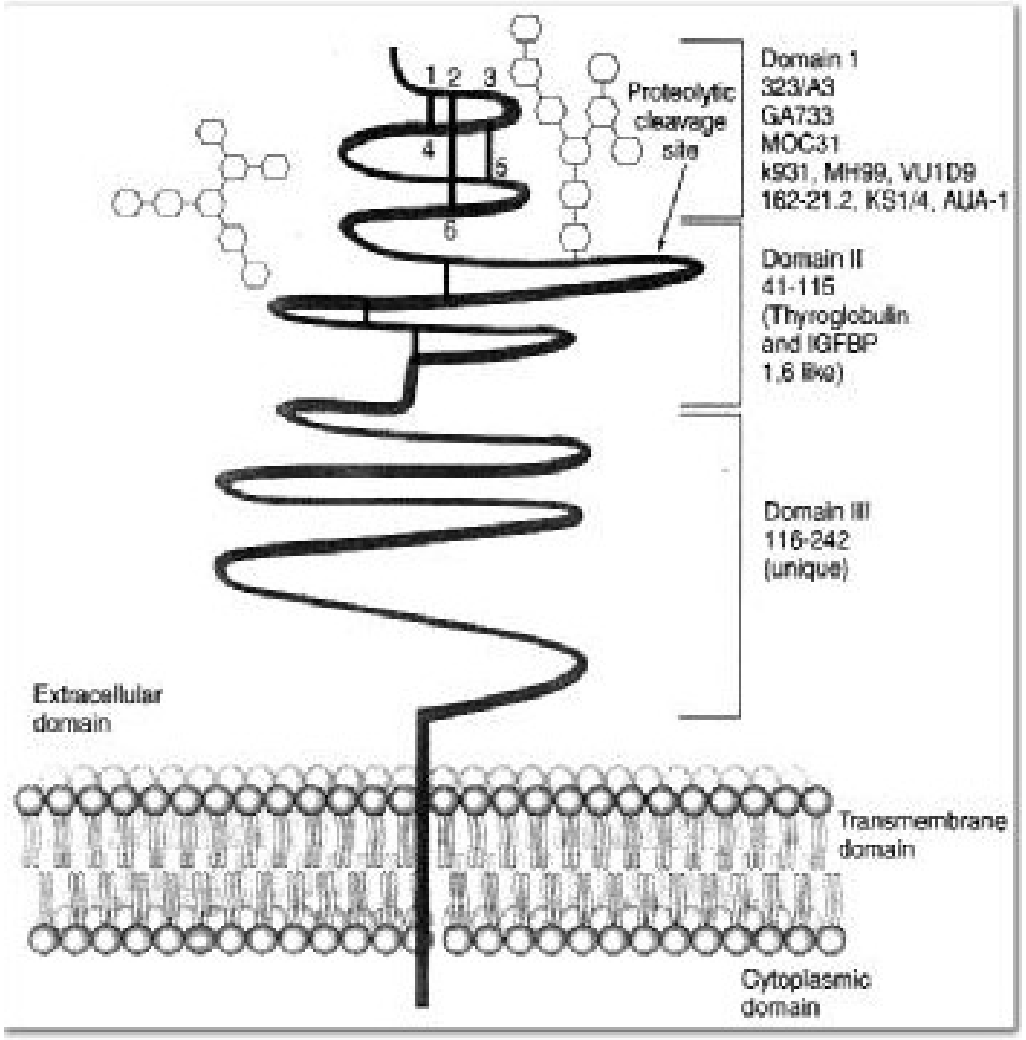
catumaxomab

Removab ®

•trifunctional rat-mouse hybrid IgG_2 monoclonal antibody specifically directed against the epithelial cell adhesion molecule (EpCAM) and the CD3 antigen

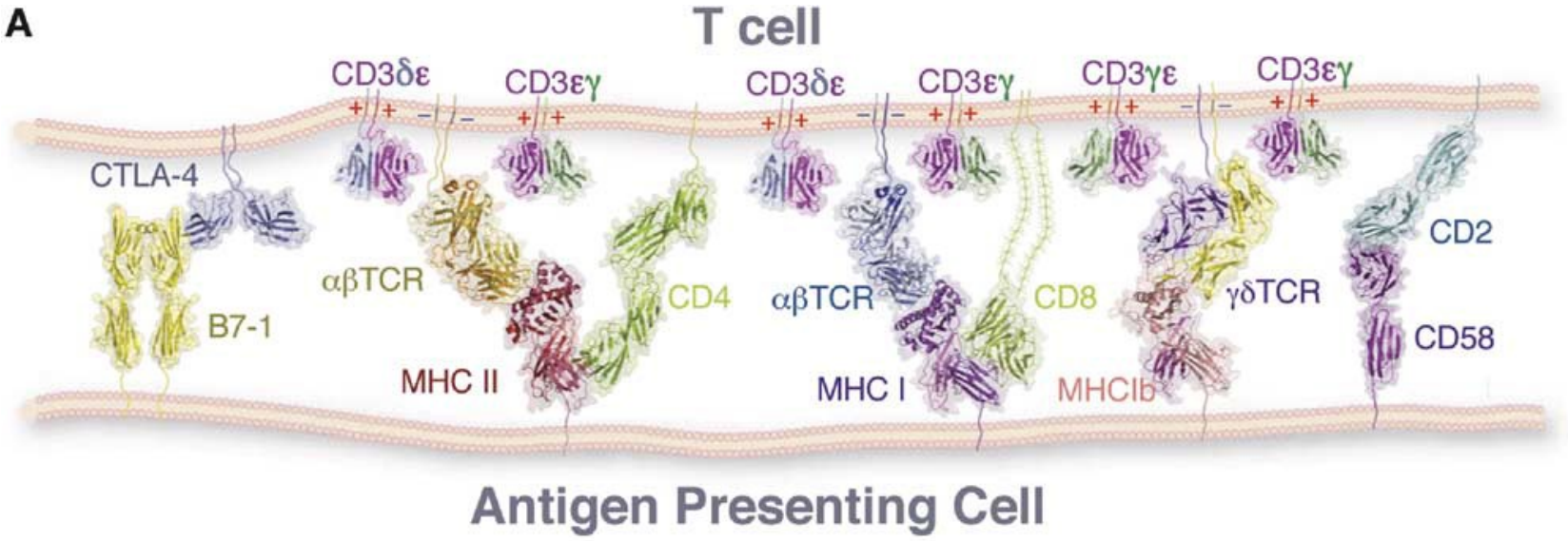
• produced in a rat-mouse hybrid-hybridoma cell line

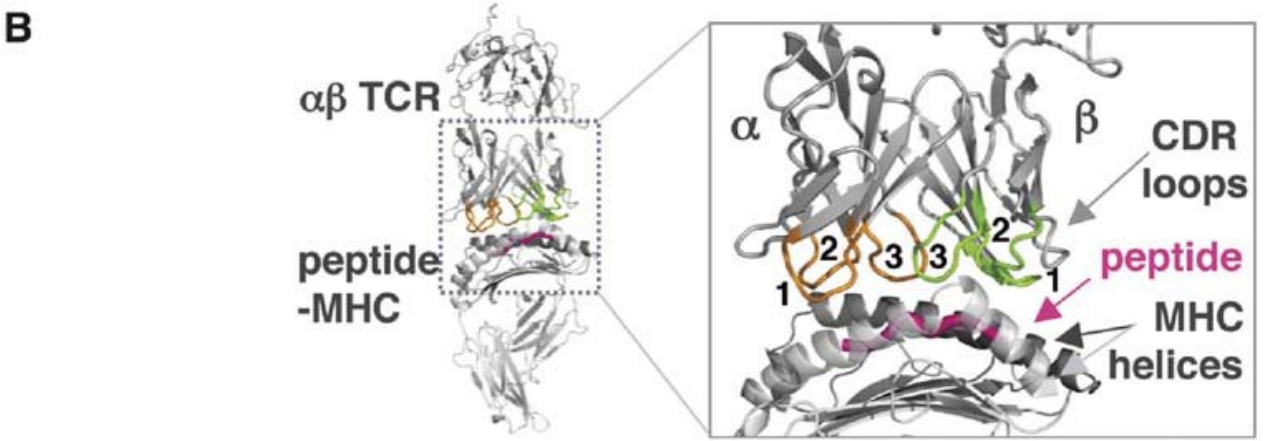
•EpCAM: a superficial glycoprotein of human cells expressed in some „normal“ ones, but namely in cancer cells („pancarcinomatic“ antigen – discovered 1979; in nearly u 100 % ovary carcinomas resistant to chemotherapy)



Tertiary structure of EpCAM containing 3 extracellular domains with cysteine -S-S- bridges and sites of glycosylation

•CD3 is one of the signal transducing modules expressed on mature T-cells
•a component of the T-cell receptor, which is a multimeric cell-surface complex and is normally responsible for the recognition of antigen associated with the major histocompatibility complex (MHC)
•if a ligand such as catumaxomab is bound to CD3, the T cell is stimulated to perform its effector functions (cytokine release and/or cytotoxicity)





Structure and mechanism of action of catumaxomab

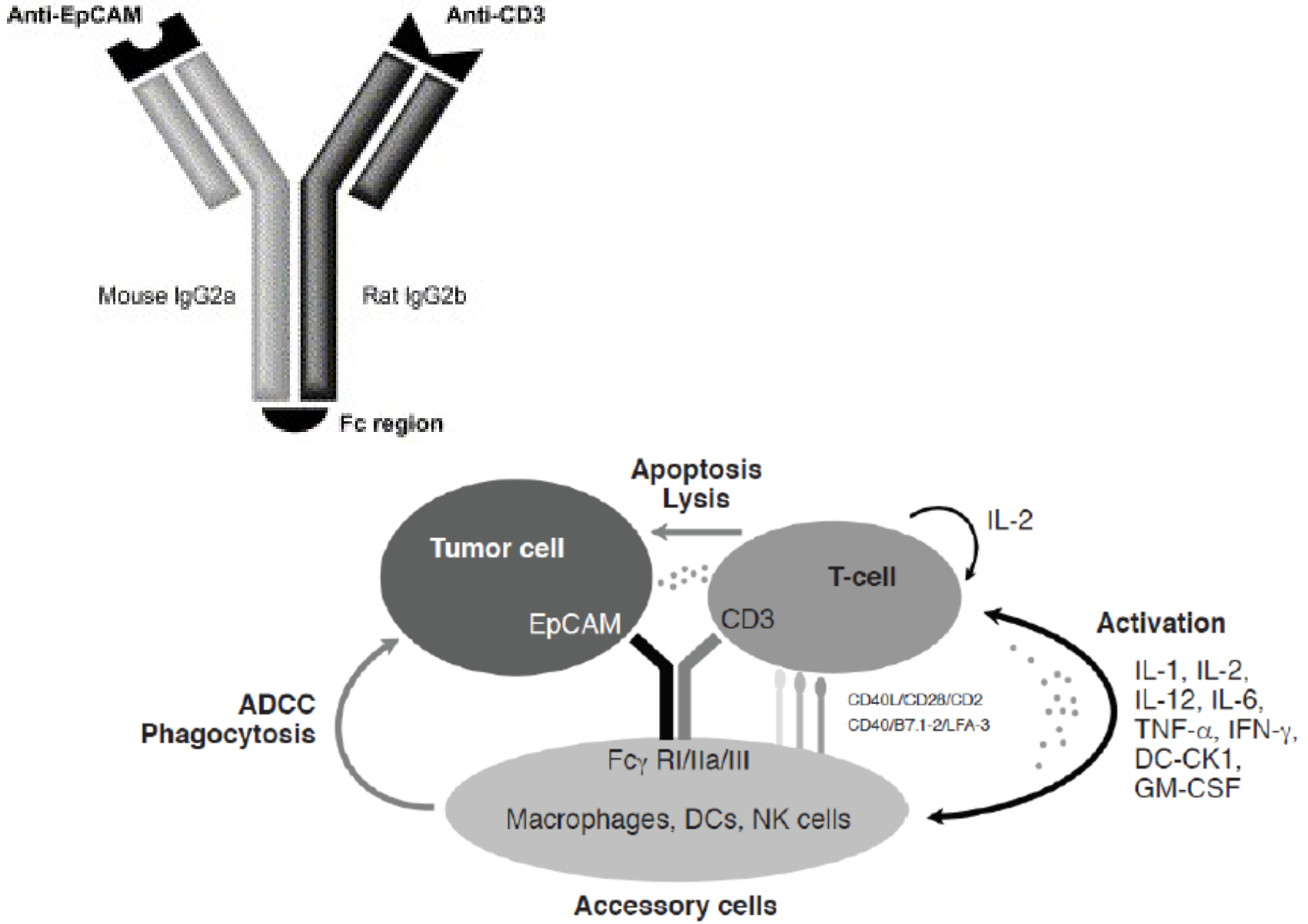


Figure 1. Catumaxomab mechanism of action. ADCC = antibody-dependent cell-mediated cytotoxicity, CK = cytokine, DC = dendritic cell, EpCAM = epithelial cell adhesion molecule, Fcγ R = Fcγ receptor, GM-CSF = granulocyte-macrophage colony-stimulating factor, IL = interleukin, IFN = interferon, LFA = lymphocyte function-associated antigen, NK = natural killer, TNF = tumor necrosis factor.

- the anti-tumour activity of catumaxomab has been demonstrated *in vitro* and *in vivo*.
- effective catumaxomab-mediated killing of tumour cells *in vitro* was observed for target cells with low and high expression of the EpCAM antigen, independent of the primary tumour type
- the *in vivo* anti-tumour activity of catumaxomab was confirmed in an immunologically compromised mouse model of ovarian carcinoma, where tumour development was delayed by an intraperitoneal treatment with catumaxomab and human peripheral blood mononuclear cells
- catumaxomab is indicated for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas where standard therapy is not available or no longer feasible

adecatumumab

syn. MT201

- human IgG_1
- against epitelial cell adhesion molecule (EpCAM) only
- phase II clinical tests against liver metastases of any cancer was proceeding from March 2009 to November 2011
- single-agent adecatumumab therapy has shown dose- and target-dependent clinical activity in EpCAM-positive MBC, albeit no objective tumor regression. Further investigation of adecatumumab in patients with EpCAM-overexpressing tumors and lower tumor burden are proposed.

ipilimumab

Yervoy ®

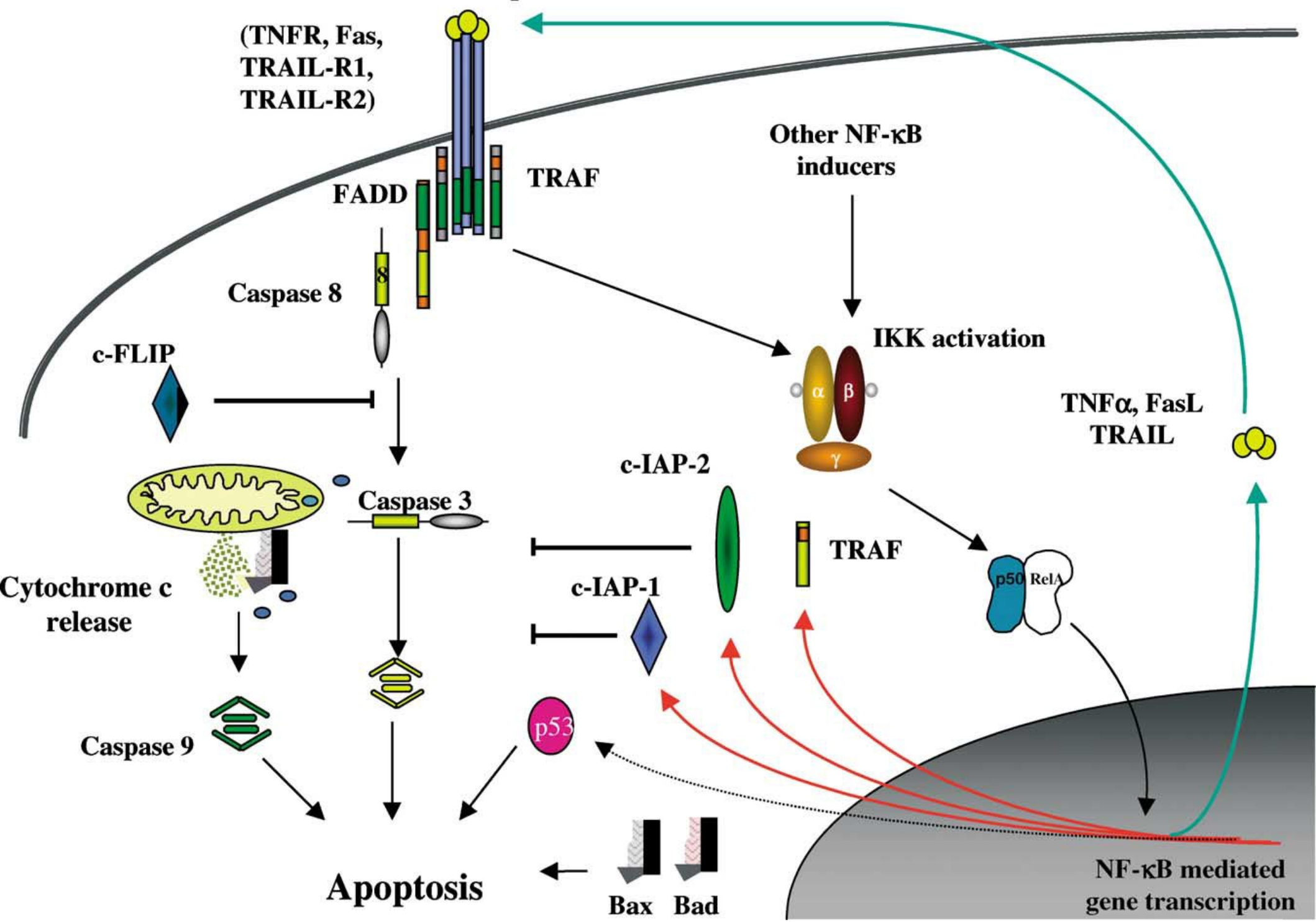
- fully human anti-CTLA-4 monoclonal antibody ($IgG_1\kappa$)
- produced in Chinese hamster ovary cells by recombinant DNA technology
- cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a negative regulator of T-cell activation
- ipilimumab is a T-cell potentiator that specifically blocks the inhibitory signal of CTLA-4, resulting in T-cell activation, proliferation, and lymphocyte infiltration into tumours, leading to tumour cell death
- the mechanism of action of ipilimumab is indirect, through enhancing T-cell mediated immune response
- indicated for the treatment of advanced (unresectable or metastatic) melanoma in adults who have received prior therapy

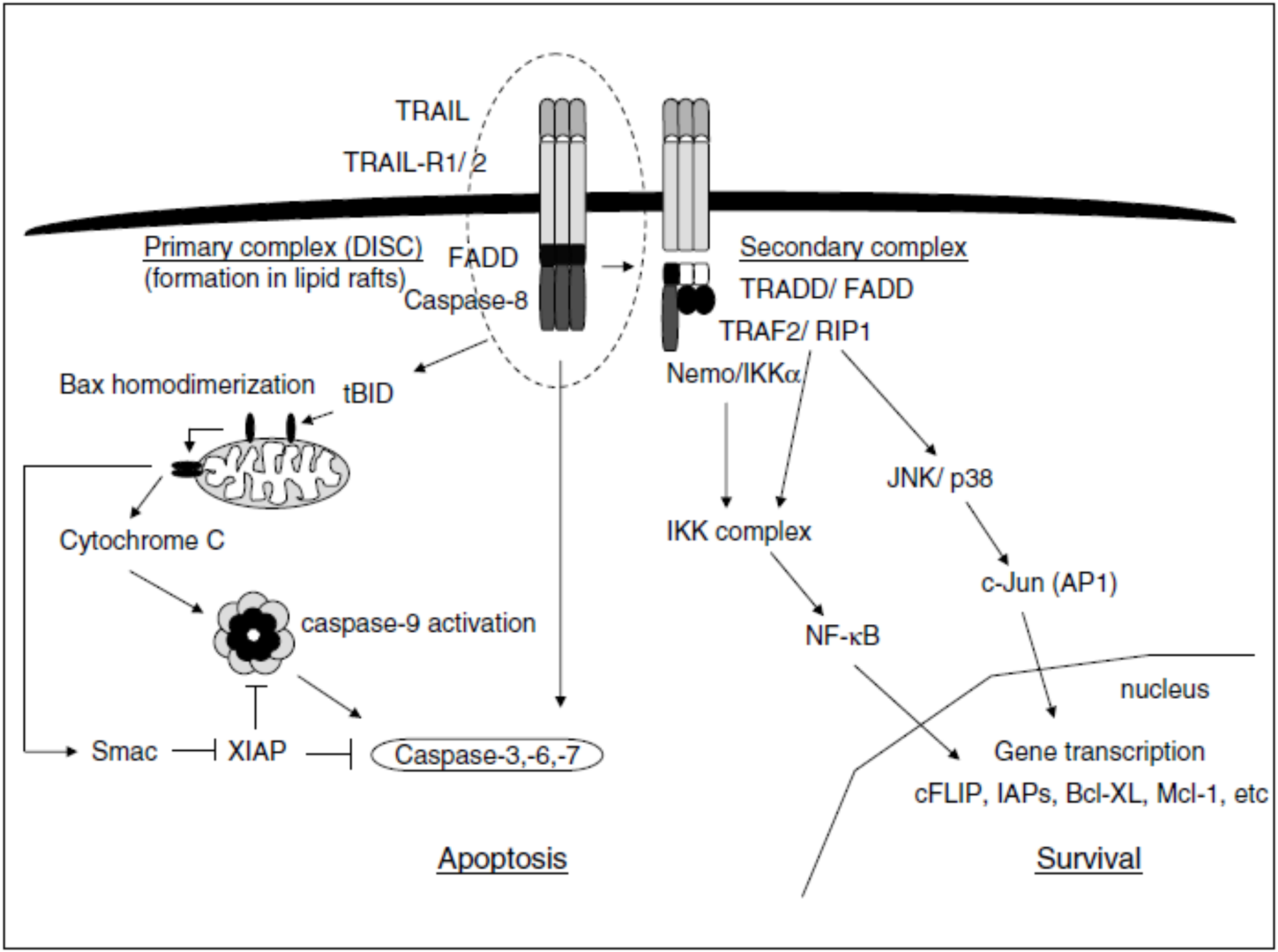
lexatumumab

- IgG_1 human monoclonal antibody – TRAIL (=Tumor necrosis factor-related apoptosis-inducing ligand) agonist

•TRAIL induces apoptosis in tumorigenic and transformed cell lines (cultures), but rarely in normal cells

•TRAIL interacts with the specific domain death receptors, DR4 and DR5, and triggers trimerization and formation of a primary complex (DISC = Death Inducing Signaling Complex) in the intracellular lipid rafts in the cytoplasm. DISC synthesis involves the recruitment of caspase 8, mediated by the adaptor proteins, into the death domain of the activated receptor. Activated caspase 8 triggers the extrinsic apoptic pathway by the direct activation of efectors, e.g. caspases 3 and 7. Except of it, caspase 8 can initiate the intrinsic apoptic pathway be the activation of pro-apotic Bid protein. Both pathways lead to caspase 3 activation and to (tumour) cell death.


(TNFR, Fas,
TRAIL-R1,
TRAIL-R2)
Other NF-κB
inducers
TRAF
FADD
Caspase 8
c-FLIP
IKK activation
α
β
γ
TNFα, FasL
TRAIL
c-IAP-2
Caspase 3
TRAF
Cytochrome c
release
c-IAP-1
p50
RelA
Caspase 9
p53
Apoptosis
Bax
Bad
NF-κB mediated
gene transcription


TRAIL
TRAIL-R1/ 2
Primary complex (DISC)
(formation in lipid rafts)
FADD
Caspase-8
Secondary complex
TRADD/ FADD
TRAF2/ RIP1
Nemo/IKKα
Bax homodimerization
tBID
Cytochrome C
caspase-9 activation
Smac
XIAP
Caspase-3,-6,-7
IKK complex
JNK/ p38
c-Jun (AP1)
NF-κB
nucleus
Gene transcription
cFLIP, IAPs, Bcl-XL, Mcl-1, etc
Apoptosis
Survival

•lexatumumab was intended for therapy of urinary bladder and prostate cancer and in this indication successfully tested in rabbits

•clinical tests of the phase I n childern and young adoults with solid carcinoma were being performed between December 2006 and December 2011

•the primary aim was to determine the tolerance of the adult maximum tolerated dose and dose limiting toxicities of lexatumumab in patients with refractory pediatric solid tumors.

•the secondary aim was to quantify tumour response to lexatumumab

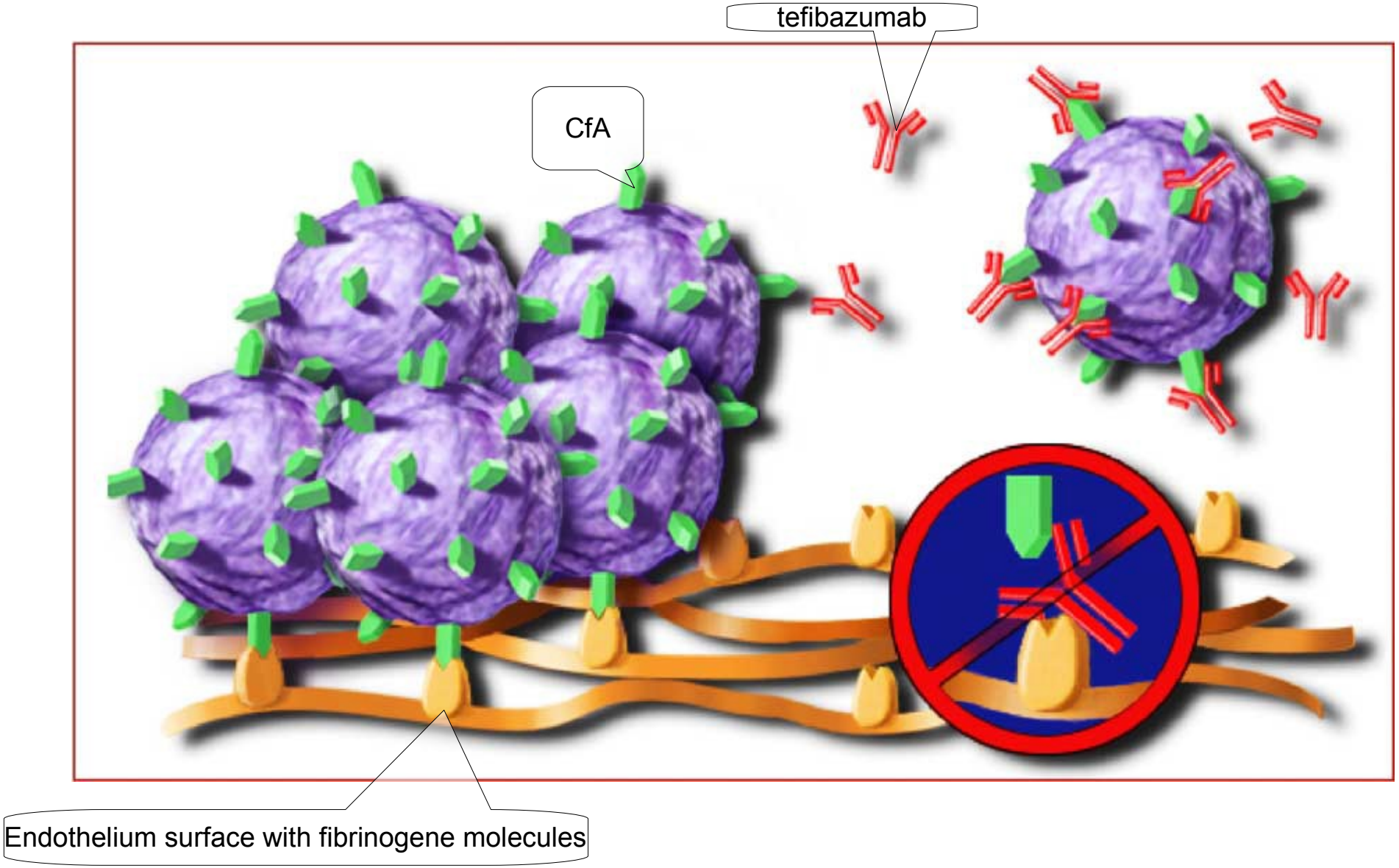
Antiinfective mabs

tefibazumab

Aurexis®

- humanized IgG_1
- M_r ~ 147 590
- treatment of *Staphylococcus aureus* infections
- targets fibrin-binding surface epitope of the clumping factor A (CfA) expressed on the surface of most *Staphylococcus aureus* strains
- CfA belongs among surface adhesins of the cell of *S. aureus* of the MSCRAMMs type (microbial surface components recognizing adhesive matrix molecules), which are a family of cell surface staphylococcal adhesins whose primary function is to facilitate bacterial attachment to the extra-cellular matrix of the host during the colonization phase of infection, and which also can facilitate colonization of implants and damaged endothelial surfaces
- CfA binds fibrinogen

Proposed mechanism of action for MSCRAMM® protein antibodies; inhibition of microbial adhesion and subsequent immune clearance, or phagocytosis



•phase II clinical tests against staphyloccoccal bacteriaemia were succesfully finished

•in June 2006, Inhibitex was seeking to outlicense certain development rights to the drug. Pending the outcome of partnering discussions, Inhibitex suspended the initiation of any additional clinical trials of tefibazumab.

•recently was Inhibitex acquired by Bristol-Myers Squibb (February 14th)

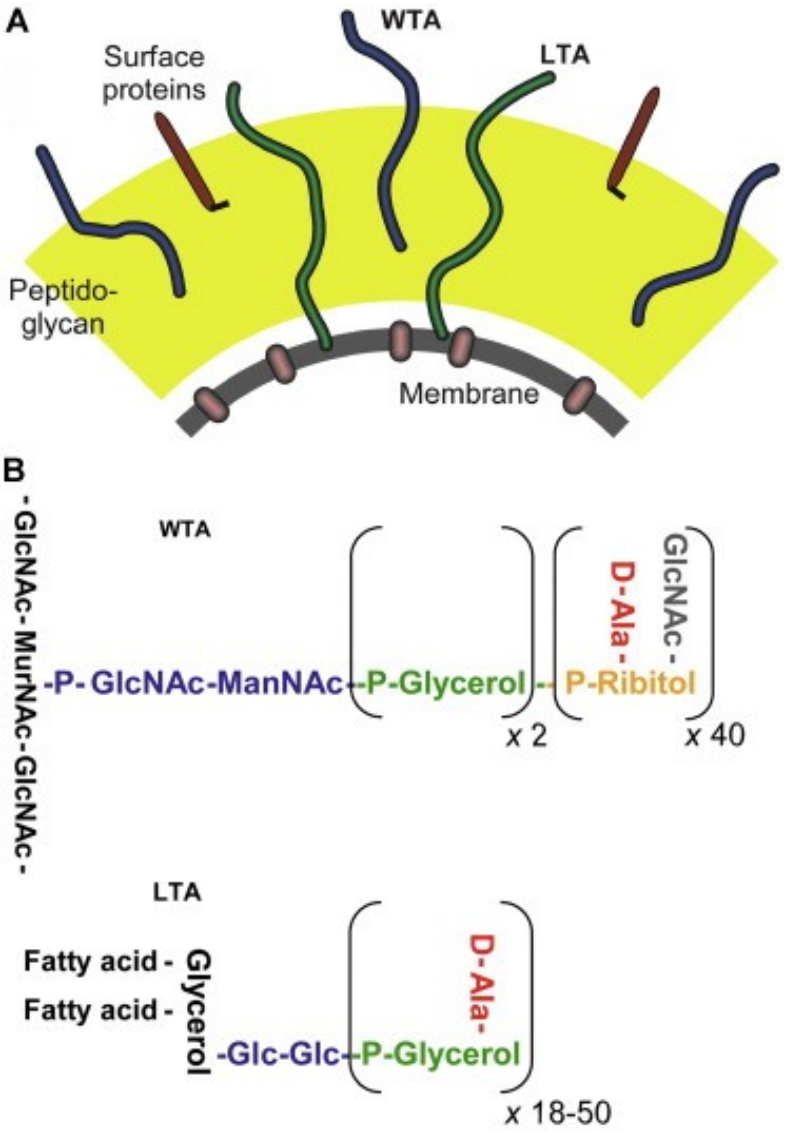
pagibaximab
syn. BSYX-A110

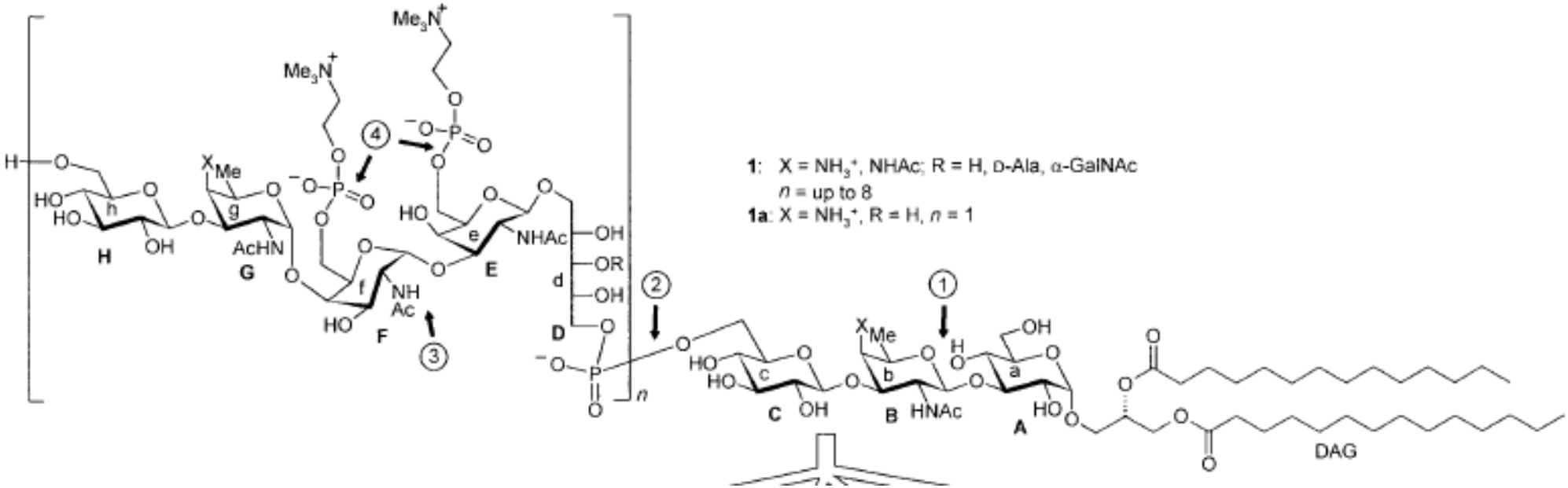
•chimeric

•against staphylococcal lipoteichoic acid (LTA) – an important constituent of the cell wall of staphylococci; LTA is anchored in the cell membrane with its lipophilic part; it inhibits bacteria phagocytosis *in vitro*, induces cytokines cascade and is supposed to be necessary for staphylococci survival, also helps staphylococci to permeate blood-brain barrier (BBB)

•prevention of staphylococcal sepsis in premature neonates with very low birth weight

•security and efficacy has been verified by phase 2 – 3 blinded clinical study: Three once-a-week 90 or 60 mg/kg pagibaximab infusions, in high-risk neonates, seemed safe and well tolerated. No staphylococcal sepsis occurred in infants who received 90 mg/kg.





Structure of lipoteichoic acid from *Streptococcus pneumoniae*

raxibacumab

ABthrax ®

- human
- $IgG_{1\lambda}$ against the „protective antigen“, which is a part of anthrax toxin
- succesfully tested in rabbits and monkeys infected with inhaled spores of *Bacillus anthracis*
- succesfull phase 3 clinical tests in healthy volunteers: a „security study“
- raxibacumab has demonstrated efficacy in a post-exposure setting even after toxins were released into the bloodstream
- in the U.S. Strategic National Stockpile (SNS) as a tool for safeguarding citizens against anthrax bioterorism attack

edobacomab
syn. E5

•mouse

•IgE against bacterial endotoxin

•endotoxin = lipopolysaccharide part of the outer membrane of bacteria; primarirly iniiates „gramnegative sepsis“: triggers the cascade of pro-inflammatory cytokines (eg. TNF), which in its result stimulates several pro- and anti-inflammatory cascades $\Rightarrow$ systemic signs typical for sepsis

•a compound binding (= neutralizing) an endotoxin could be capable to stop this cascade

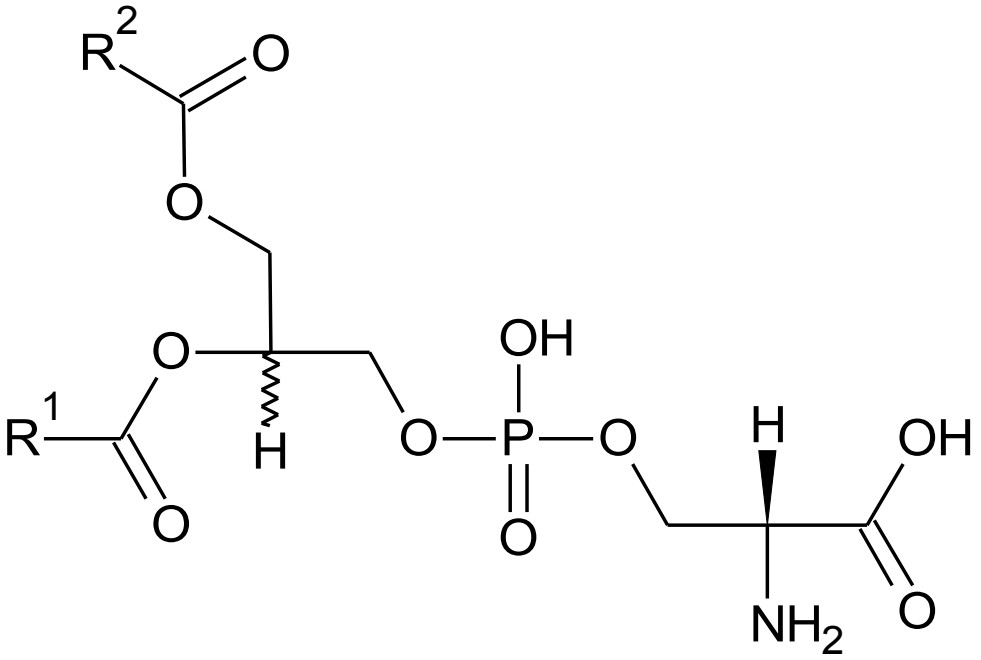
•endotoxins of various G^- bacteria markedly differ one from each other, E5 is however bound to the „nucleus“ - a sequence that is common for all

•phase 3 clinical study: E5 did not improve short-term survival compared with placebo

bavituximab

Tarvacin ®

- chimeric
- against phosphatidylserine (PS) bound to β_2-glycoprotein I
- phosphatidylserine – the most widely spread anionic phospholipide of the plasmatic mebrane



Phosphatidylserine

•PS is normally tightly segregated to the internal surface of the plasma membrane in most cell types, including the vascular endothelium

•PS asymmetry is maintained by ATP-dependent aminophospholipid translocases (Mg^{2+}-ATPases) that catalyze the transport of aminophospholipids from the external to the internal leaflet of the plasma membrane

• loss of PS asymmetry occurs during apoptosis, necrosis, cell activation and transformation, resulting in the exposure of PS on the external surface of the cells

•similar situation occurs in viruses infected cells, in which replication proceeds: activation of the cell by a virus causes intracellular Ca^{2+} loss $\Rightarrow$ triggering of PS „exporteurs“ & inhibition of its „import“ inside the cell by means of translocases $\Rightarrow$ „externalisation“ of PS

•potential anti-tumour and antiviral drug (HCV, HIV)

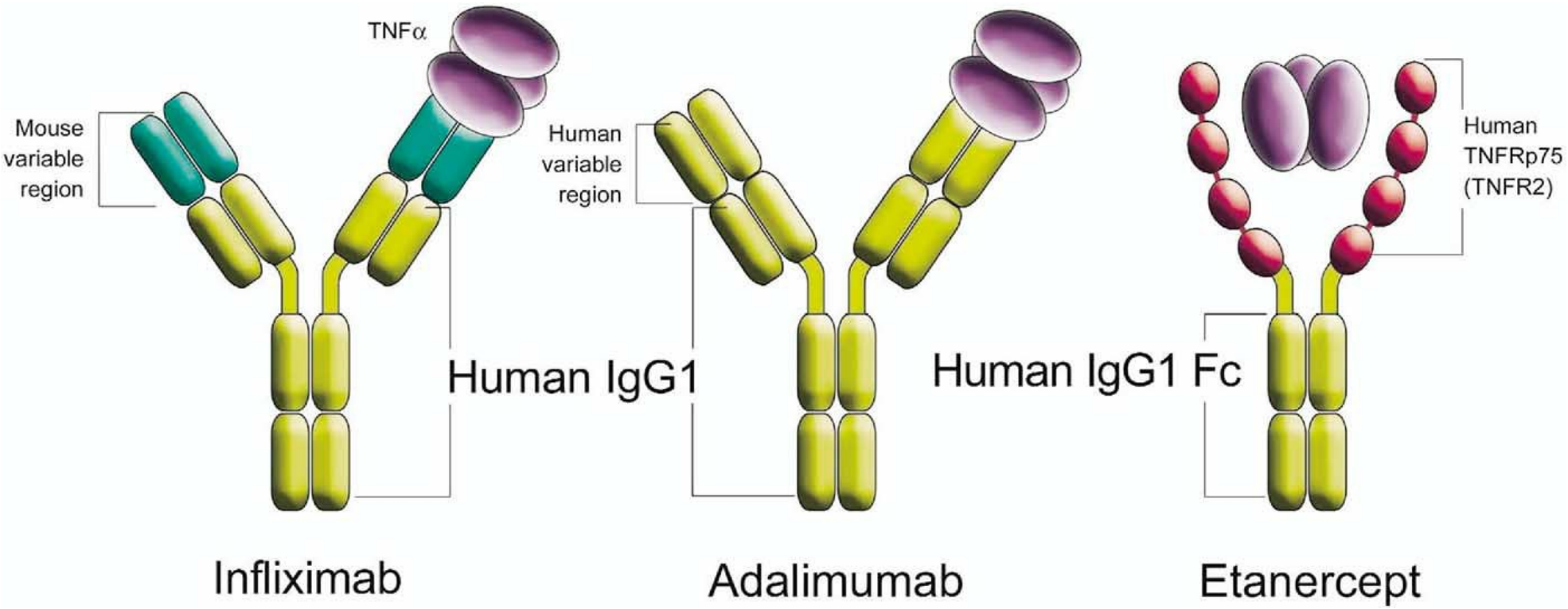
•approx. 15 finished and ongoing phase 1 and 2 clinical studies against hepatitis C and/or HIV infection and various tumours (breast, non-small cell lung, liver, prostate, pancreatic) in USA, India, Georgia, Russia and Ukraine

Drugs for chronic inflammatory diseases

Tumour Necrosis Factor

There are two types of tumour necrosis factor: TNF -α and TNF - β . Of the two, TNF - α has been studied in more detail. TNF - α is a 157 amino acid polypeptide. It is a mediator of immune regulation, including the activation of macrophages and induction of the proliferation of T cells. Another TNF - α function is its cytotoxic effects on a number of tumor cells. Recent research, however, concentrates on its property in the stimulation of inflammation, particularly in the case of rheumatoid arthritis, Crohn disease, ulcerative collitis etc. Clinical trials are being conducted with drugs to block TNF - α with anti - TNF - α monoclonal antibodies. These antibodies target the excessive levels of TNF - α in the synovial fluid of joints or in the intestinal mucosa and provide relief to sufferers of rheumatoid arthritis or of an inflammatory disease of GIT.

TNF-α inhibitors



„Anti-TNF molecules“ - are bound to TNF and neutralize its activity

Infliximab (Remicade ®) : mouse/human chimaera, where variable regions of murine antibody are linked to constant regions of human IgG_1

Adalimumab (Humira ®): recombinant human antibody of IgG_1 type expressed in Chinese Hamster Ovary cells

Golimumab (Simponi ®): recombinant human antibody of IgG_1 type produced by a murine hybridoma cell line with recombinant DNA technology

Certolizumab pegol (Cimzia ®): recombinant, humanised antibody Fab' fragment against TNF expressed in *Escherichia coli* and conjugated to polyethylene glycol (PEG).

Etanercept (Enbrel ®): soluble dimeric fusion protein in which human p75 TNF receptor is linked to Fc domain of human IgG_1

Usage: treatment of rheumatoid arthritis, inflammatory intestinal disease (ulcerative colitis, Crohn disease...) and many other inflammatory diseases.