Biologics or biological drugs

•officially (WHO) „biological and biotechnological substances“

Basic characterization of biologics

•typically acquired by other way than by classical chemical synthesis (semisynthetic modifications are possible)

•typically M_r > 1000 (up to 1000 „small molecules“) - greater, more complex, usually exhibit a *primary structure* (a sequence of amino acids or nucleotides), a *secondary structure* (α-helix, “folded sheet”, influence of -S-S- bridges), a *tertiary structure* (general space arrangement of a monomeric molecule) and a *quraternary structure* (grouping of monomers); many proteins are glycosylated

•but both above conditions need not be necessarily fulfilled for classification of a drug as a biologic

Some possible problems in terminology

•pharmaceutics = technology of manufacturing of application forms of drugs

("pharmaceutical technology" is the literal translation from Czech)

⇓

•biopharmaceutics ≈ biopharmacy = „a discipline concerning drug absorption" on a frontier of pharmaceutics and pharmacokinetics (pharmacology)

•biologicals: analogy to chemicals ⇒ they include „biological drugs" but also diagnostic monoclonal antibodies, enzymes used in technology etc.

•biologics: the term mostly used for „biological drugs"

⇓

„Biopharmaceuticals" is not a perfect name for a subject devoted to "biological drugs."

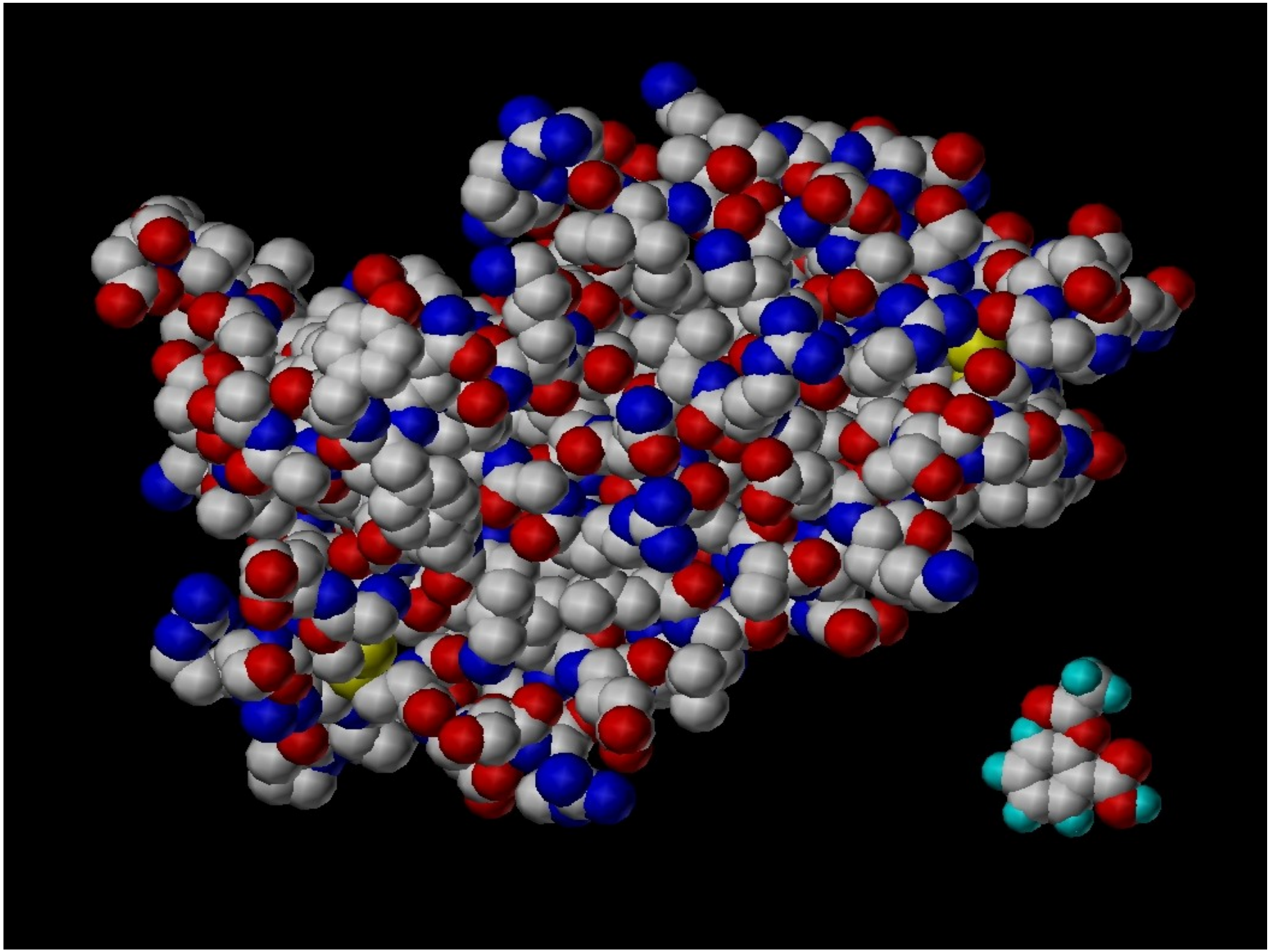
⇒maybe "biological therapies" or "biological treatment"

Differences in production of “small molecules” and biologics

•small molecules – classical organic synthesis: chemicals with exactly defined chemical structure and purity react under exactly defined conditions with a predictable and preciously verifiable results

•biologics- preparation by „harvesting“ of compounds produced ancreted by artificially constructed cells (genetic engineering)

An illustration of the difference between a biologic and a “small molecule”
erythropoietin and acetylsalicylic acid



History of biologics

•the Antiquity and the Middle Ages: usage of leeches for treatment of circulation and blood disorders (hirudin)

•classical vaccines: preparation of dead or attenuated bacterial cultures or attenuated or inactivated viruses (e.g. pox: transfer of the infection „from a skin to a skin“ has been known long since around 1000 A.D. in China; 1796 – Edward Jenner demonstrated that putting of the purulence from a furuncle of the cowpox in under the skin protected against the infection with pox; 1805 – 1st vaccine against pox was prepared on the calf skin in Italy; 1864 – mass usage of this vaccine; after 1940 – lyofilized vaccines (Collier))

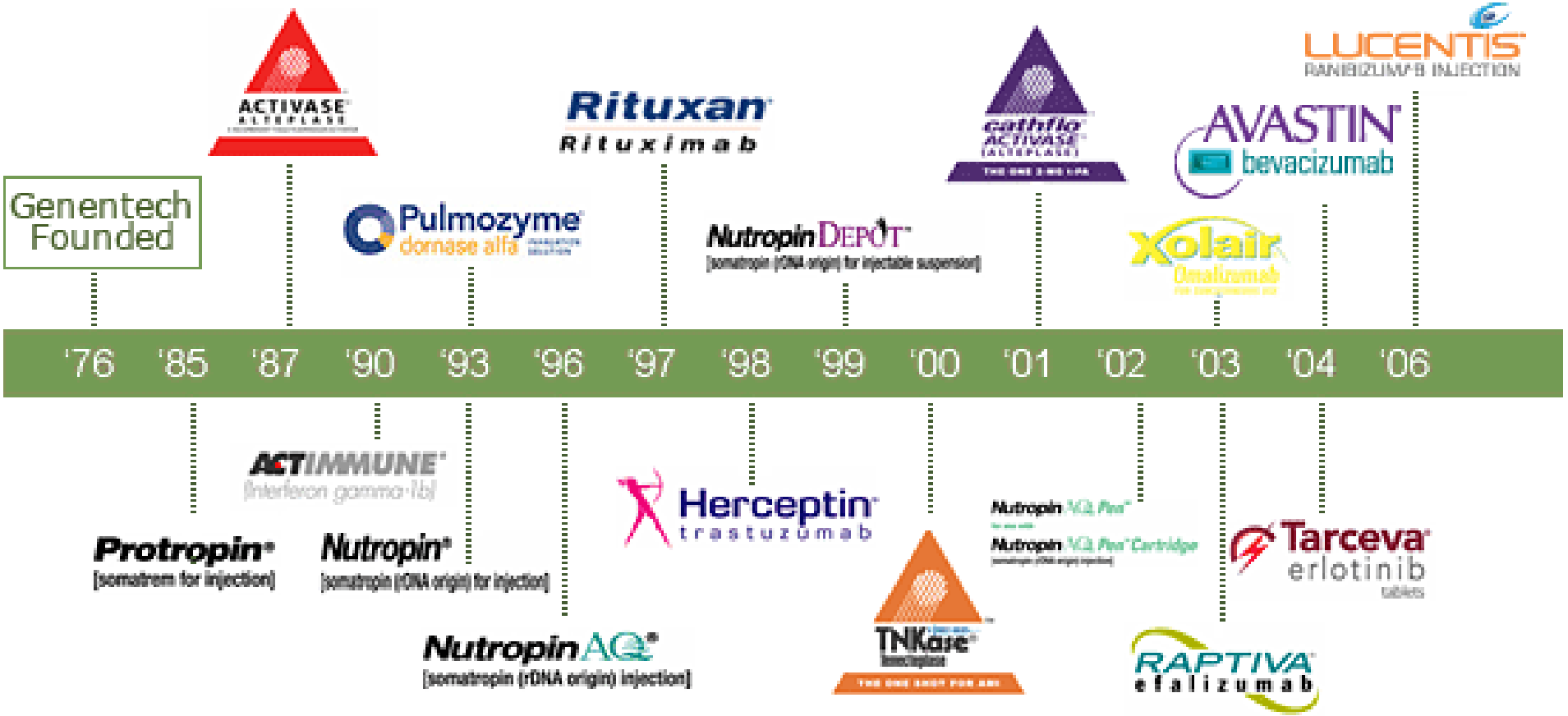
•(poly-clonal) antibodies („sera“) - immunisation of a suitable production macro-organism (eg. horse, rabbit) with a noxious agent (a toxin, e.g. a snake poison), a serum acquired from the blood used as an antidote; monoclonal antibodies for analytic and diagnostic purposes, then a suitable transformation (RIA, ELISA)

•peptides – isolation from biological material (insulin: Banting and Best 1921)

More recent history of biologics – genetic engineering

- 1977 - somatostatin first time prepared by a recombinant technology in *E. coli* (Genetech, USA)
- 1978 – human insulin cloned
- 1982 – recombinant human insulin prepared in *E. coli* marketed
- 1984 – Factor VIII of the blood clotting first time prepared in a laboratory
- 1985 – FDA approved somatrem, a somatotropin analogue

History of biologics from the point of view of a corporation (Genetech)



Development and authorisation of biologics

EMA (EU): normal approval procedure like for any other drug; approvals of biosimilars possible

FDA (USA): possibility of taking part into so called Fast Track Drug Development Program (since 1998, revised 2004) – the prerequisite is the usefulness for serious or life endangering condition and legitimated hope for better clinical efficacy than up to the present time used drugs; approvals of biosimilars also possible

- "common cluster for biosimilars": EU (EMA) + USA (FDA) + Canada + Japan

Generics and „biosimilars“

Generics: small molecules – contain the same active compound as the original and reach 80 – 105 % of the bioavailability of the original

„Biosimilars“ or „Follow-up Proteins“: contain a biologic prepared by the similar procedure and with the similar effects as the original

Different approaches of FDA (USA) and EMA (EU)

- EMA: biosimilars started to be approved since 2007
- FDA: since 2015

Pharmacological classification of biological and biotechnological substances in accordance with WHO

• Drugs for alimentary tract and metabolism: insulins.

•Anti-infectives: antimicrobial, bactericidal permeability increasing polypeptides, human papilomavirus.

•Antineoplastics: peptide vaccines, recombinant vaccines, toxins.

•Blood and agents acting on the heamopoietic system: antithrombins, blood coagulation cascade inhibitors, blood coagulation factors, erythropoietin type blood factors, heparin derivatives including low molecular mass heparins (heparinoids), hirudin derivatives, trombomodulins.

•Immunomodulators and immunostimulants: colony stimulating factors, inteferons, interleukin receptor antagonists, interleukin type substances, monoclonal antibodies , receptor molecules, native or modified, tumor necrosis factor antagonists.

•Hormones, hormone antagonists, hormone-release stimulating peptides or hormone-release inhibiting peptides (excluding insulins): growth hormon (GH) derivatives, its antagonists, oxytocin derivatives, pituitary / placental glycoprotein hormones, pituitary hormone-release stimulating peptides, synthetic polypeptides with cortikotropin-like action, vasoconstriktors, vasopressin derivatives.

•Various: „antisense“ oligonucleotides, enzymes, gene therapy products, growth factors, peptides a glycopeptides not classified above.

Basics of biologics nomenclature in accordance with WHO recommendations

[INTERNATIONAL NONPROPRIETARY NAMES (INN) FOR BIOLOGICAL AND BIOTECHNOLOGICAL SUBSTANCES (A REVIEW) INN Working Document 05.179 08/11/2007]

2.1 Groups with respective stems

Name of the group	Stem
antisense oligonucleotides	*-rsen*
blood coagulation cascade inhibitors	*-cogin*
blood coagulation factors	*-cog*
colony stimulating factors	*-stim*
enzymes	*-ase*
erythropoietin type blood factors	*-poetin*
growth factors	*-ermin*
growth hormone derivatives	*som-*
heparin derivatives including low molecular mass heparins	*-parin*
hirudin derivatives	*-irudin*
hormone-release inhibiting peptides	*-relix*
interleukin receptor antagonists	*-kinra*
interleukin type substances	*-kin*
monoclonal antibodies	*-mab*
oxytocin derivatives	*-tocin*
peptides and glycopeptides (for special groups of peptides see *-actide, -pressin, -relin, -tocin*)	*-tide*
pituitary hormone-release stimulating peptides	*-relin*
receptor molecules, native or modified (a preceding infix should designate the target)	*-cept*
synthetic polypeptides with a corticotropin-like action	*-actide*
tumor necrosis factor antagonists	*-nercept*
vasoconstrictors, vasopressin derivatives	*-pressin*

Basics of biologics nomenclature in accordance with WHO recommendations (continued)

2.2 Groups with respective pre-stems

Name of the group	Pre-stem
antimicrobial, bactericidal permeability increasing polypeptides	*-ganan*

2.3 Groups with INN schemes

Name of the group
antithrombins
gene therapy products
insulins
interferons
pituitary / placental glycoprotein hormones

Basics of biologics nomenclature in accordance with WHO recommendations
(continued)

2.4 Groups without respective stems / pre-stems and without INN schemes

Name of the group
growth hormone antagonists
human papilloma virus
peptide vaccines / recombinant vaccines
thrombomodulins
toxins

3.6 General policies for monoclonal antibodies [1] [3]

- The common stem for monoclonal antibodies is *-mab*.
- Sub-stems for source of product:

a	rat
axo (pre-sub-stem)	rat-murine hybrid
e	hamster
i	primate
o	mouse
u	human
xi	chimeric
zu	humanized

Chimeric vs. humanized monoclonal antibodies

The distinction between chimeric and humanized antibodies is as follows:

A chimeric antibody is one that contains contiguous foreign-derived amino acids comprising the entire variable region of both heavy and light chains linked to heavy and light constant regions of human origin.

A humanized antibody has segments of foreign-derived amino acids interspersed among variable region segments of human-derived amino acid residues and the humanized heavy-variable and light-variable regions are linked to heavy and light constant regions of human origin.

Nomenclature of monoclonal antibodies – continued

•a **sub-stem** for disease or target class is situated before a sub-stem for source of the product:

- Sub-stems for disease or target class:

-ba(c)-	bacterial
-ci(r)-	cardiovascular
-fung-	fungal
-ki(n)- (pre-sub-stem)	interleukin
-le(s)-	inflammatory lesions
-li(m)-	immunomodulator
-os-	bone
-vi(r)-	viral

Nomenclature of monoclonal antibodies – continued

tumours:

-co(l)-	colon
-go(t)-	testis
-go(v)-	ovary
-ma(r)-	mammary
-me(l)-	melanoma
-pr(o)-	prostate
-tu(m)-	miscellaneous

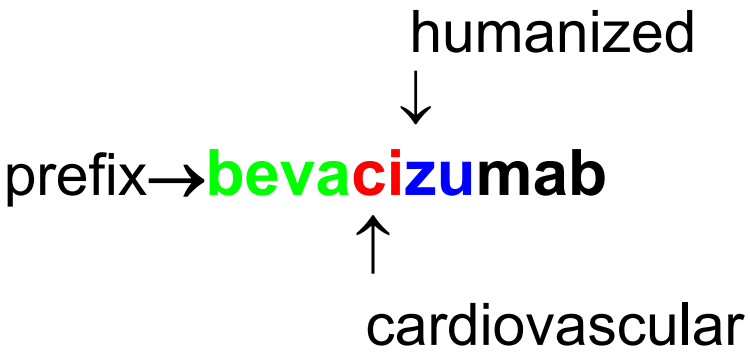
Whenever there is a problem in pronunciation, the final letter of the sub-stems for diseases or targets may be deleted, e.g. *-vi(r)-*, *-ba(c)-*, *-li(m)-*, *-co(l)-*, etc.

Nomenclature of monoclonal antibodies – continued

Prefix

Should be random e.g. the only requirement is to contribute to a euphonious and distinctive name.

An example of the INN name of a monoclonal antibody



Examples of particular biologics: monoclonal antibodies

Antineoplastics

ramucirumab

syn. IMC-1121B

•humanized

•angiogenesis inhibitor

•targeted against VEGFR-2 receptor

•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

•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.

bevacizumab

Avastin®

•chimeric: 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)

•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

cetuximab

Erbitux®

•chimeric

•blocks receptors for epidermal growth factor (EGFR)

•family of receptors for epidermal growth factor includes 4 structurally similar receptors: Erb/HER (EGFR): HER-1 or ERBB1, EGFR-2 (HER-2 or ERBB2), HER-3, and 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

•cetuximab approved by both FDA and EMA for treatment of metastasising colorectal carcinomas expressing EGFR

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

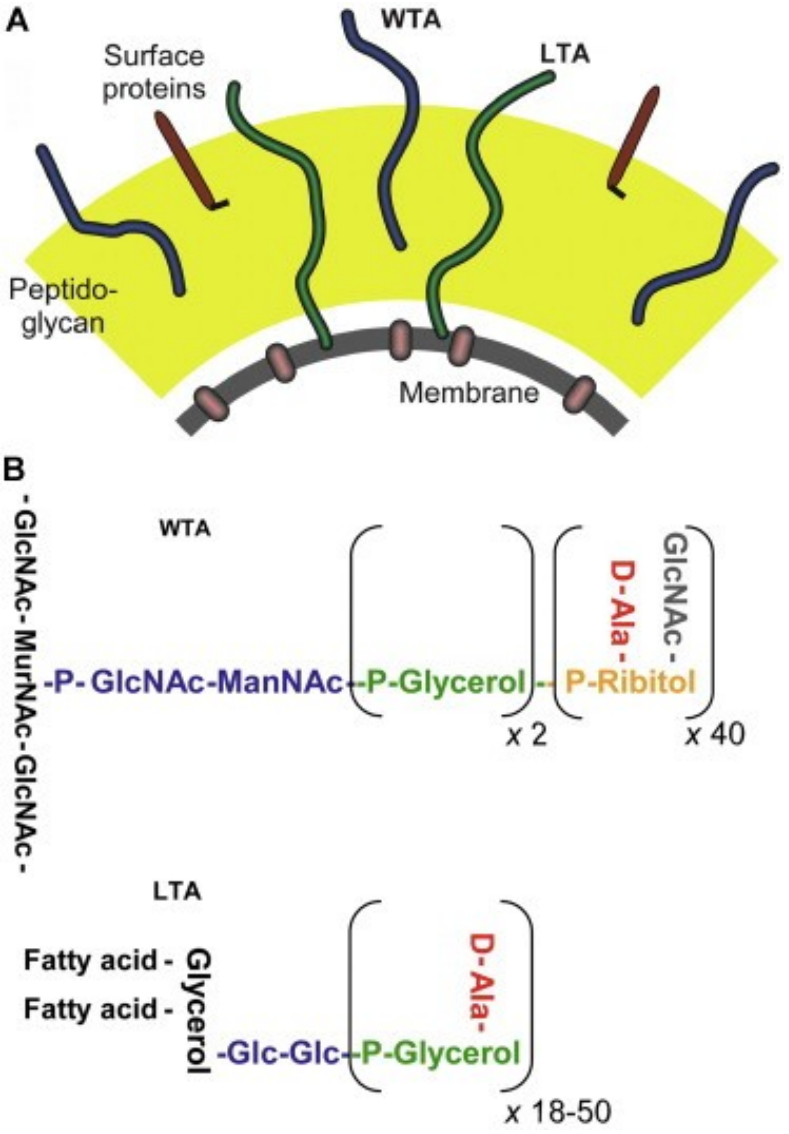
Antibacterial compounds

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 – efficacy verified by phase 2 – 3 blinded clinical study



Antiviral drugs

bamlanivimab

Bamlanivimab ®

syn. LY3819253

- recombination neutralization human
- IgG_1 against spike protein of SARS-CoV-2 virus
- blocks binding of the spike protein to the human ACE2 receptor, a thus avoids the following entrance of the virus into human cells and replication of the virus
- F_c fragment not modifies; it has full effector functions of the antibody.
- not approved; in ČR used based on the permission in accordance with § 8 article 6 of the Act 378/2007 Coll. about drugs; in EMA, an accelerated registration procedure „the rolling review“ is ongoing; in USA, approved by FDA in the EUA regimen (Emergency Use Authorizations)

Indications: alone or with etesevimab (= “antibody cocktail”) for treatment of the confirmed disease COVID-19 in patients older than 12 years who who do not require therapeutic administration of oxygen due to disease COVID-19 and who are at high risk of progression to severe COVID-19 disease

- should only be used in an environment where physicians have immediate access to medications to treat a severe infusion reaction, such as anaphylaxis
- the state of patients is observed during the administration and at least one hour after finishing of the infusion

$\Rightarrow$ indicated (= prescribed) by a general practitioner, but must be administered in a hospital (to non-hospitalized patients)

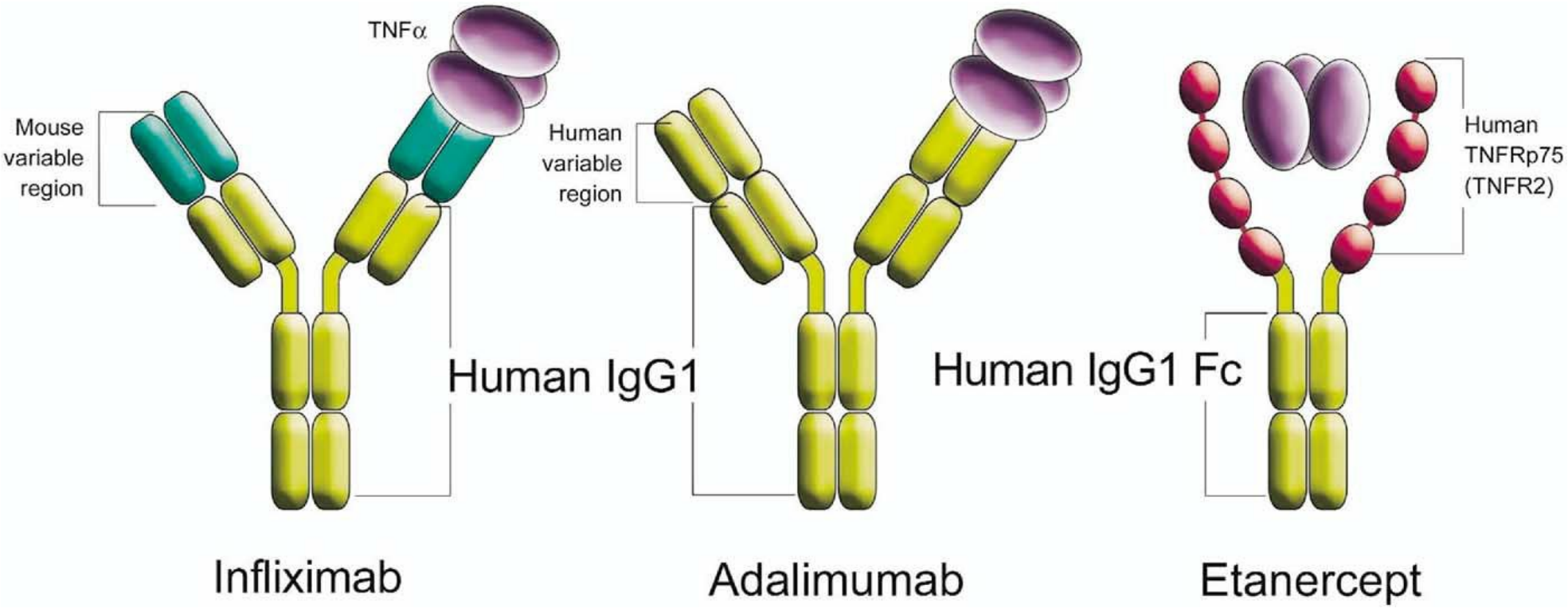
etesevimab
syn. LY3832479 or LY-CoV016
Etesevimab ®

- recombination neutralization human
- IgG_1 against spike protein of SARS-CoV-2 virus
- blocks binding of the spike protein to the human ACE2 receptor, a thus avoids the following entrance of the virus into human cells and replication of the virus
- F_c fragment modified by substitutions of amino acids (L234A, L235A) $\Rightarrow$ effector functions reduced.
- bamlanivimab and etesevimab bind to different but overlapping epitops of receptor binding domain (RBD) of the spike protein
- not approved; in ČR used based on the permission in accordance with § 8 article 6 of the Act 378/2007 Coll. about drugs; in EMA, an accelerated registration procedure „rolling review“ is ongoing; in USA, approved by FDA in the EUA regimen (Emergency Use Authorizations)

Indications: with bamlanivomab only for treatment of the confirmed disease COVID-19 in patients older than 12 years who who do not require therapeutic administration of oxygen due to disease COVID-19 and who are at high risk of progression to severe COVID-19 disease

Drugs for chronic inflammatory diseases

TNF-α inhibitors



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

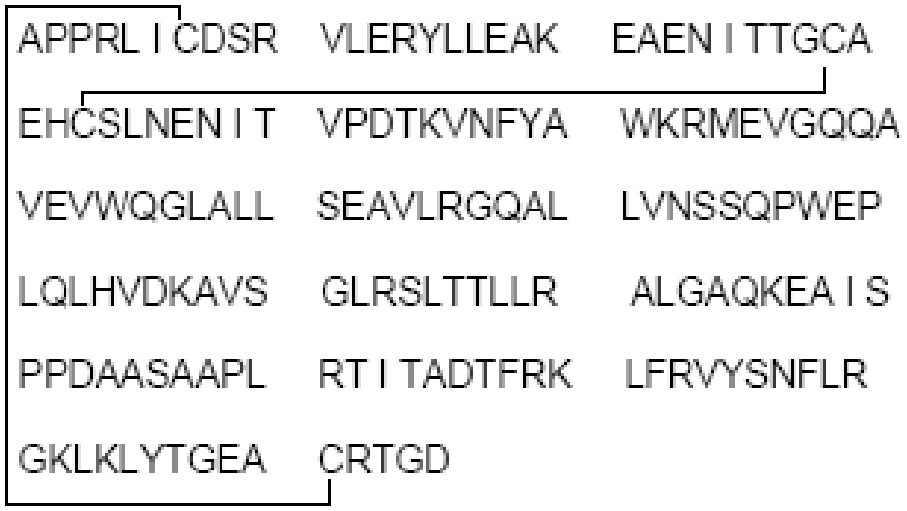
Infliximab: mose/human chimaera, where variable regions of murine antibody are linked to constant regions of human IgG_1

Adalimumab: (recombinant) human antibody of IgG_1 type

Etanercept: soluble dimeric fusion protein in which human p75 TNF receptor is linked to F_c domain of human IgG_1

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

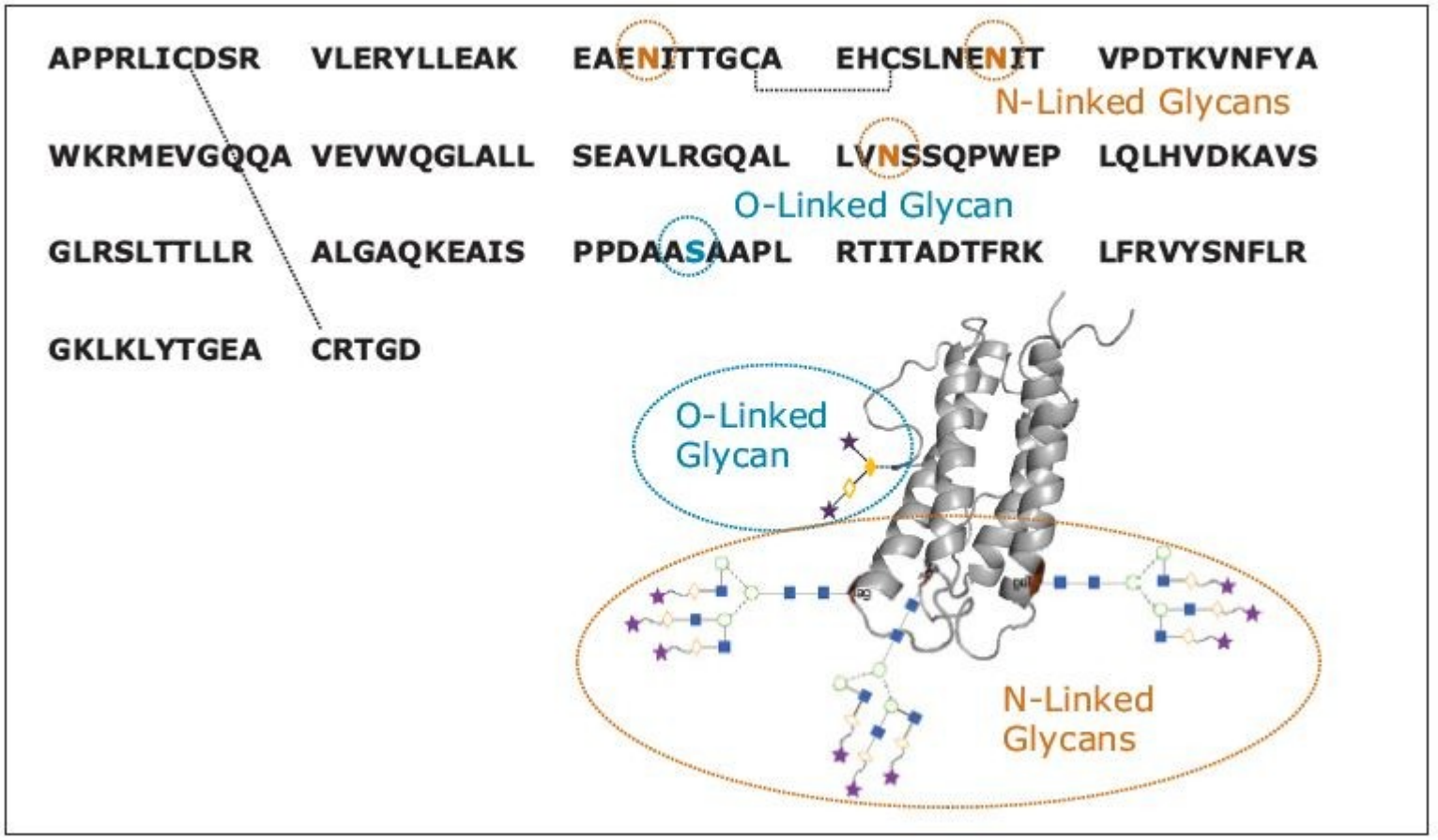
Examples of particular biologics: Blood factors of erythropoetine type



erythropoietin (EPO)
= glycosylated protein from 165 AA M_r about 30 600 CAS 113427-24-0
Erythropoietini solutio concentrata EP
= a solution containing a group of closely related glycoproteins, which are not to distinguish from the natural human erythopoietin (human urine erythropoietin, huEPO), from the point of view of 165 amino acids sequence and their average profile of glycosylation

- naturally released from kidneys of adults and in liver of foetus
- stimulates stem cells of bone marrow to proliferation and differentiation
- produced *in vitro* mostly in rodent cell lines by a method based on the recombinant DNA
- technology
- **INN names: epoetin + greek letter spelt in full** (eg. epoetin beta)
- various epoetins differ in glycosylation, complex branched oligomeric sugar chains are attached
- treatment of anaemia in chronic kidney failure, misused for doping

Epoetins´ glycosylation



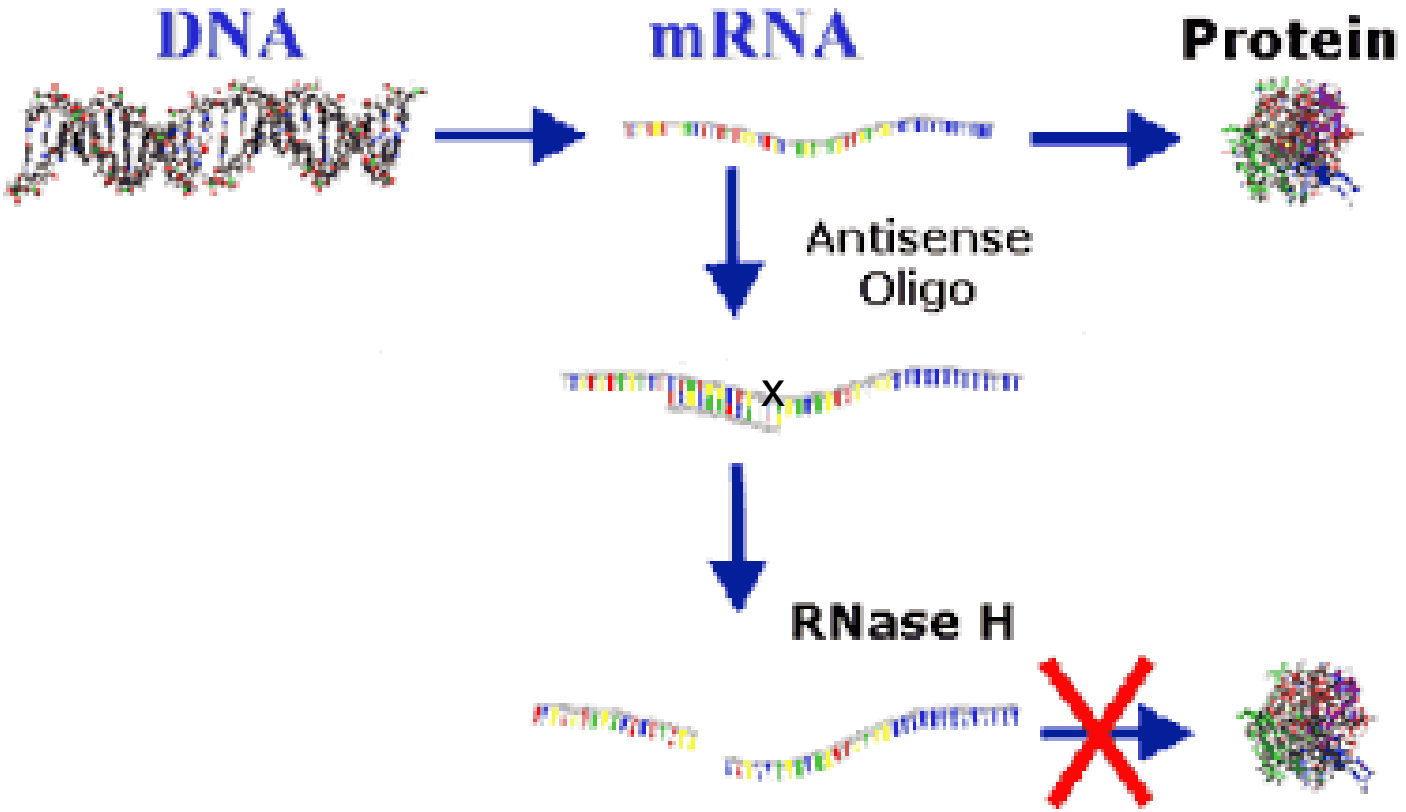
Sites of *N*-glycosylation: Asn24, Asn38, Asn83 (= N24, N38, N83)
Site of *O*-glycosylation*:* Ser126 (= S126)

Overview of epoetins

INN name: epoetin	Year of discovery/approval	Production organism / tissue	M_r CAS	Glycosylation pattern	Originator product/biosimilar	Brand names ®, generic codes
alfa	2000	Chinese hamster ovary	113427-24-0	similar to uhEPO	orig/biosim	**Eprex,** Binocrit, Abseamed
beta	1997	Chinese hamster ovary	122312-54-3		orig	Neorecormon
gama	1990	C127 murine cells transfected with huEPO cRNA	28 000-31 000 130455-76-4		orig	TYB-5220
delta	2002 - 2009	human fibrosarcoma cell line HT-1080	261356-80-3	less *O*-acetyls in *O*-glycan chains; similar to uhEPO	orig	Dynepo
epsilon	1995		154725-65-2		orig	
zeta	2007	Chinese hamster ovary	32 000-40 000 604802-70-2		biosim. of EPO alfa	Silapo, Retacrit
theta	2009	Chinese hamster ovary	762263-14-9	sugars represent 40 % of total M_r	orig	Biopoin, Eporatio
kappa	2010	Chinese hamster ovary	11096-26-7		biosim. of EPO alfa	Epoetin alfa BS injection ®

Examples of particular biologics: antisense oligonucleotides

•usually short complementary chains of modified RNA, which are proposed to avoid translation of a damaged or an undesirable sequence by binding to a respective region of RNA or DNA

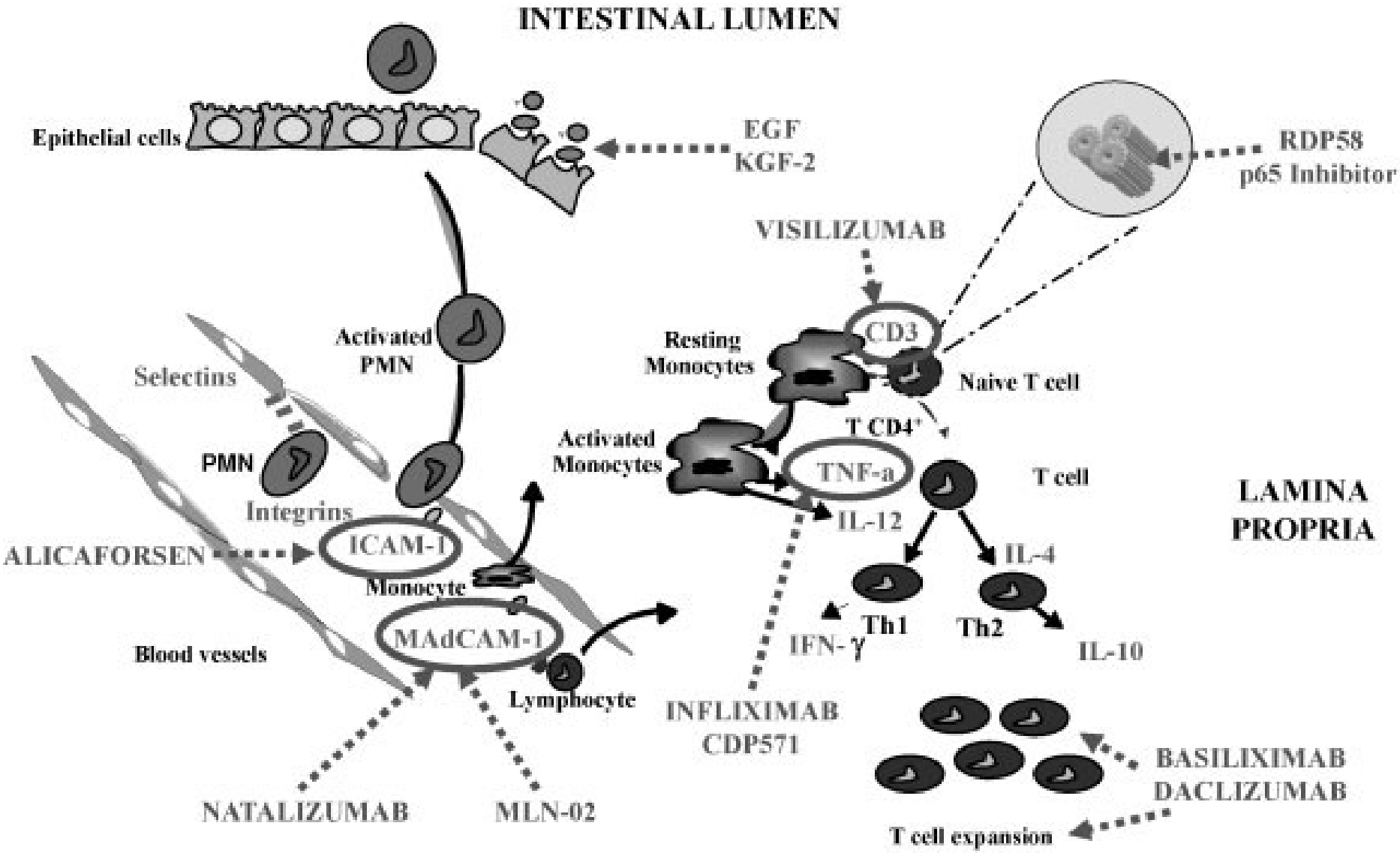


Examples of use of modified antisense oligonucleotides:

Intercellular adhesion molecule (ICAM) inhibitors

Ulcerative colitis = chronic relapsing inflammatory disease of the mucous layer of the intestine

- idiopathic = unknown ethiology
- pathogenesis is supposed to be multifactorial and include genetic, environmental and immunologic factors
- the chronic inflammation manifests namely due to adaptive immunity system dysregulation, which leads to a change of the tolerance of intestinal bacteria and to anomal response to normal luminal microflora $\Rightarrow$ immunologic imbalance $\Rightarrow$ $\uparrow$ production of inflammatory cytokines and **adhesion molecules** (e.g. ICAM), $\uparrow$ activation of polymorphonuclear monocytes (PMN); their migration into the intestine and interaction with the epithelium alters many functions of epithelium from the barrier one to electrolytes management



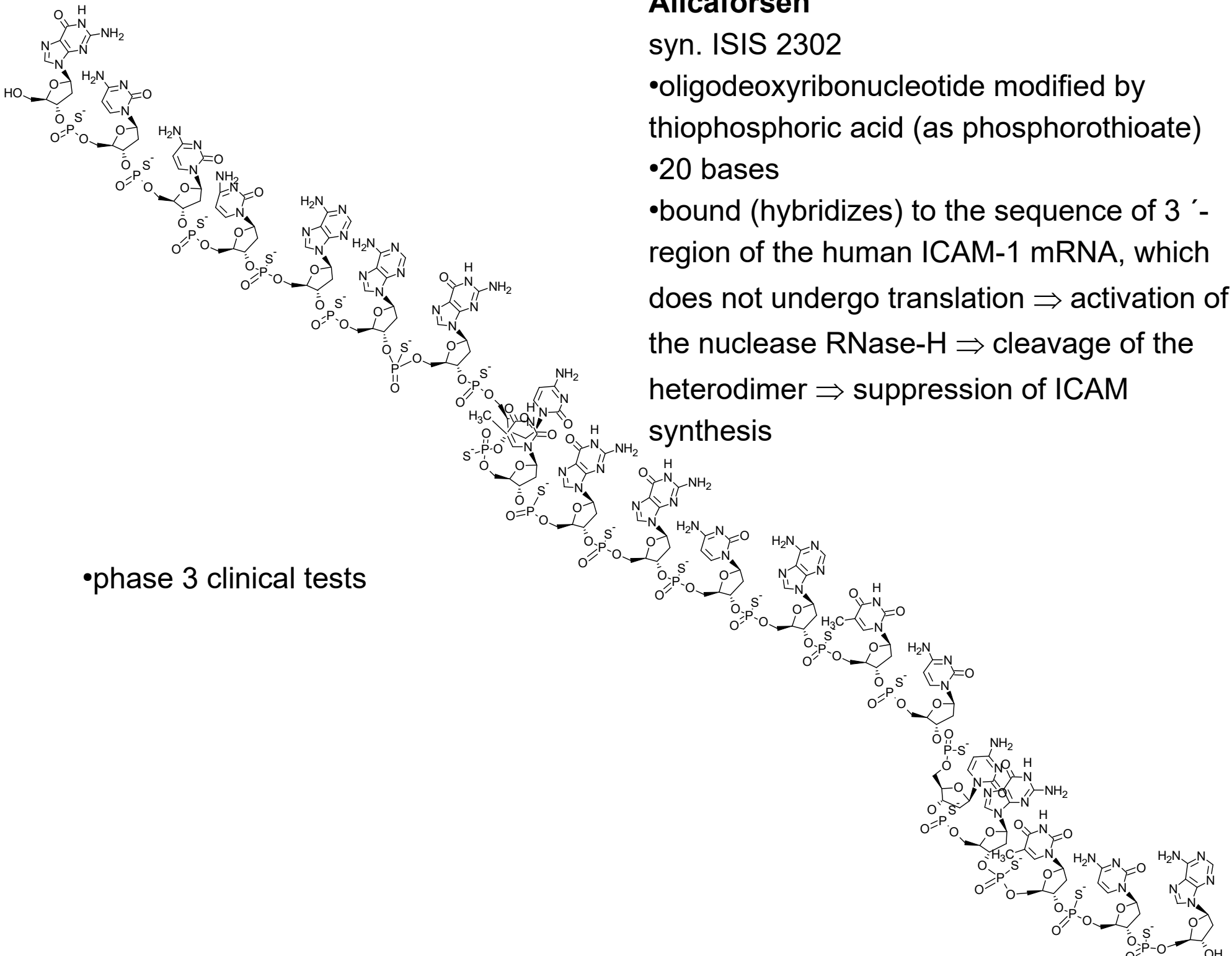
Mechanism of formation of ulcerative colitis, some biomolecules involved in them and therapeutical targets of selected biologics

ICAM-1 intercellular adhesion molecule 1 EGF epidermal growth factor

MadCAM mucose adressed adhession molecule PMN – polymorphonuclear monocyte

IFN interferon

IL interleukin



Alicaforsen

syn. ISIS 2302

•oligodeoxyribonucleotide modified by thiophosphoric acid (as phosphorothioate)

•20 bases

•bound (hybridizes) to the sequence of 3 ´-region of the human ICAM-1 mRNA, which does not undergo translation ⇒ activation of the nuclease RNase-H ⇒ cleavage of the heterodimer ⇒ suppression of ICAM synthesis

•phase 3 clinical tests

Examples of use of modified antisense oligonucleotides: antineoplastics

Oncogene Bcl-2 antagonist

•Bcl-2: antiapoptic protein; its predominance over the structurally related proapoptic Bax predisposes cancers to bad response to usual anti-cancer therapies and bad prognosis

oblimersen, G3139, augmerosen, Genasense®

•deoxyribonucleotide, 18 nucleotides, phosphorothioate, heptadecasodium salt

•T-C-T-C-C-C-A-G-C-G-T-G-C-G-C-C-A-T

•complementary to the first six codones of human Bcl-2 mRNA

•administration in *i.v.* infusion

•clinical tests of phases 1 to 3 against various types of cancers; efficient; relatively low toxicity

•Compounds in use: treatment of Duchenne muscular dystrophy (DMS)

•DMS: 1 of 3500 newborn boys is suffers from the disease, DMS results in a progressive weakening of muscles, cardiomyopathy and respiratory failure

•cause: the open reading frame and full translation of DMD gene interrupted by mutations; DMD gene encodes a peptide dystrophin

•oligonucleotides targeted to pathology splicing elements („splice switching oligonucleotides“) in DMD pre-mRNA can case skiping of exone, renewal of open reading frame and production of functional, despite truncated dytrophine chain and thus to a relief of disease symptoms

eteplirsen, Exondys 51® (approved in USA), AVI-4658 (AVI BioPharma, Portland, OR, USA)

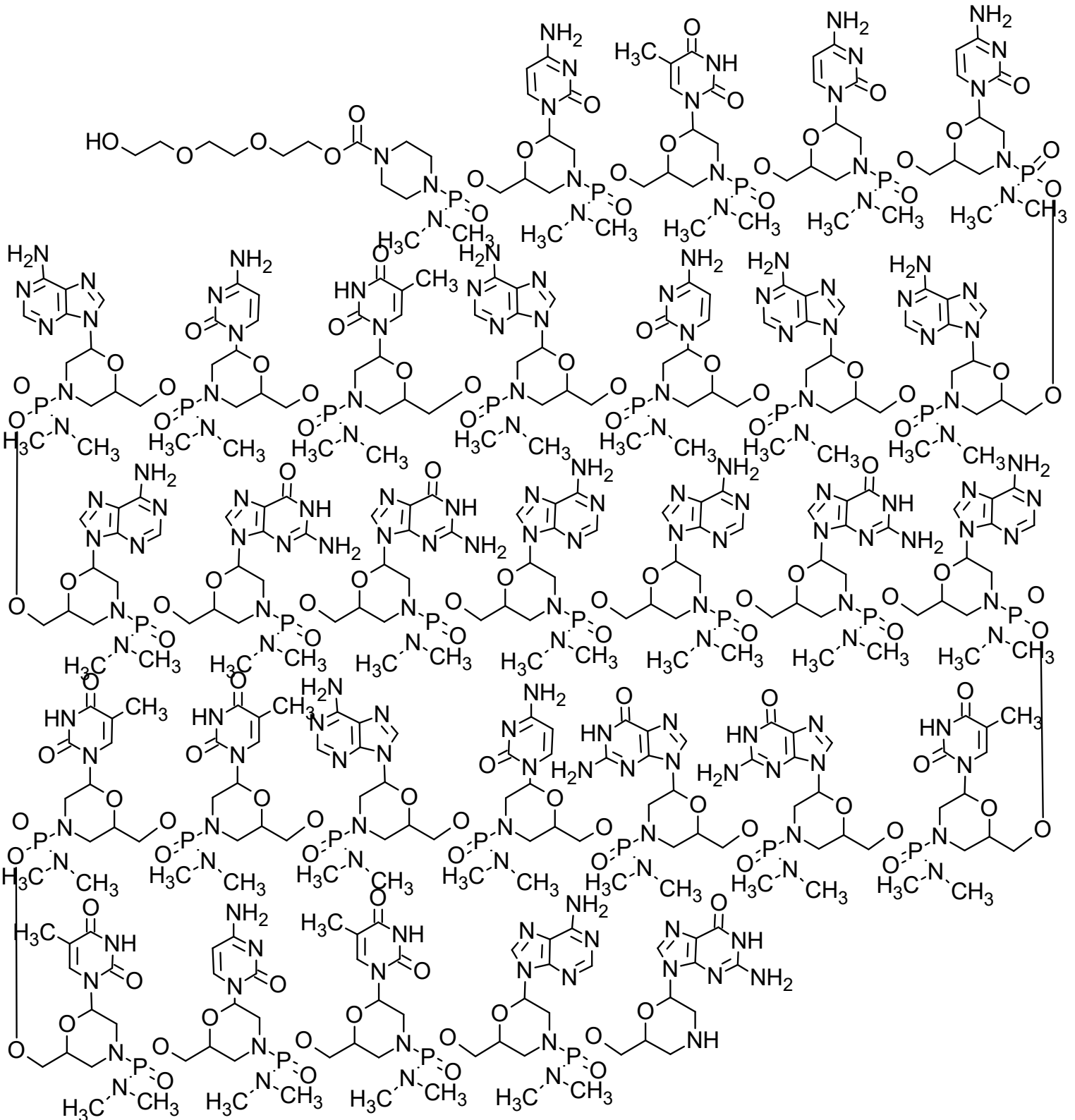
•morpholine oligonucleotide targeted to 51st exon

•Sequence CTCCAACATCAAGGAAGATGGCATTTCTAG

Exondys 51 ®

• given *i.v.*, 30 mg/kg, in 35 – 60 min long infusion once weakely

•indication: DMD treatment in u patients with confirmed DMD gene mutation, where „skiping“ of exone 51 is possible



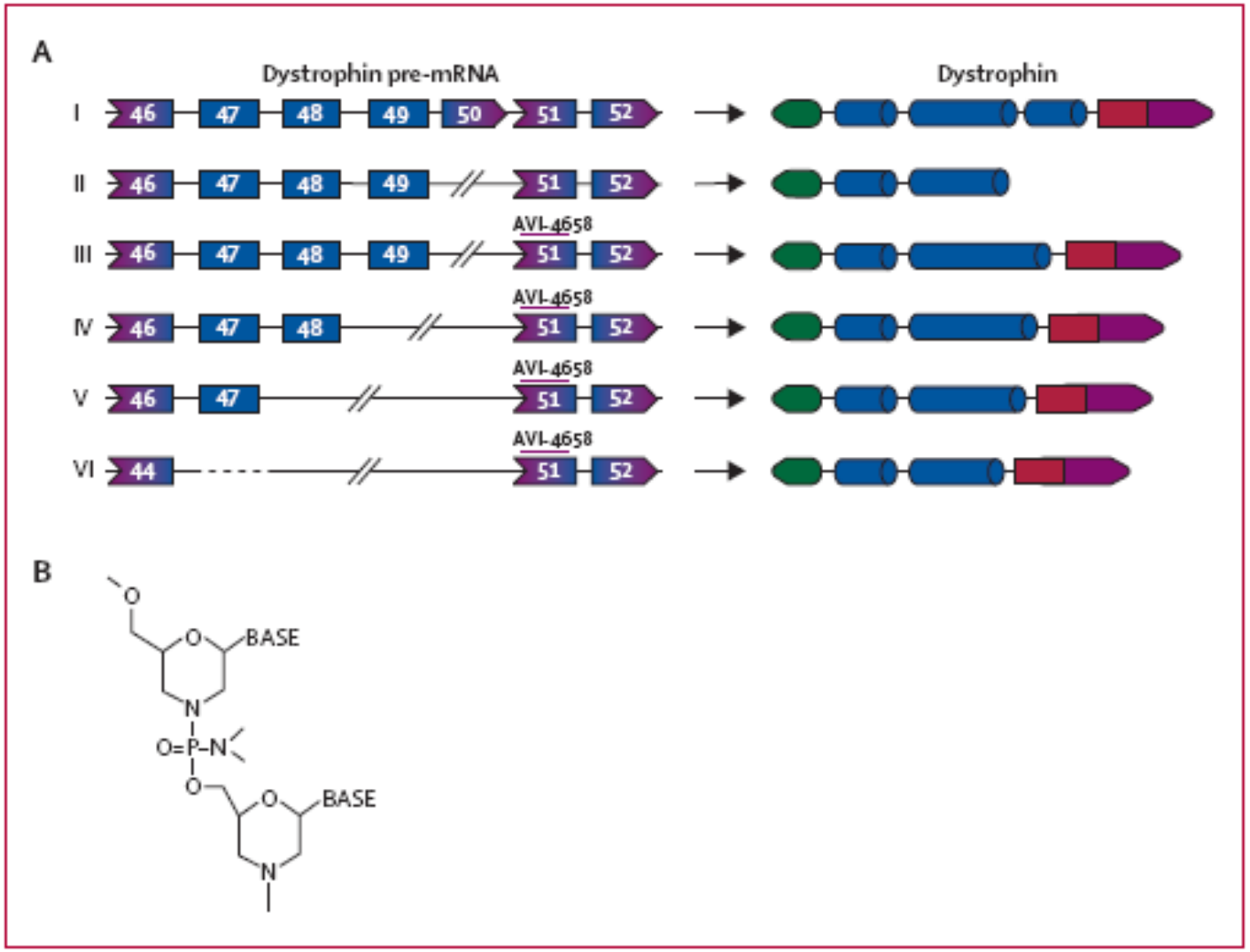


Figure 1: **Deletions and predicted results of exon skipping in the patients who were studied**
(A) Pre-mRNA transcripts and dystrophin protein products from full length *DMD*, in patients with Duchenne muscular dystrophy, and predicted protein sequences after exon skipping. (I) The normal dystrophin gene produces the full length dystrophin product. (II) Patients 1 and 2 had a deletion in exon 50 that disrupts the open reading frame, leading to a truncated and unstable dystrophin. (III) Skipping of exon 51 restores the reading frame, producing a truncated but functional dystrophin that lacks exons 50 and 51. (IV) Patient 7 is missing exons 49 and 50. (V) Patients 3 and 4 are missing exons 48–50. (VI) Patients 5 and 6 are missing exons 45–50. All the truncated dystrophins produced after skipping of exon 51 are missing the hinge 3 region and some of the rod domain but have been associated with the milder BMD phenotype.[3,10] (B) Structure of the phosphorodiamidate morpholino modification of the antisense oligomer.

Disadvantages of biologics (except adverse effects, which are in general the same as in small molecules)

- **imunogenicity** – induction of formation of antibodies against the drug
- HAMA – human anti-mouse antibodies – formed against mouse peptide sequences in chimeric biologics
- HAHA – human anti-human antibodies – formed against fully human antibodies or other biologics; bound to a unique binding site, where they are not tolerated by the immunity system
- neutralizing × non-neutralizing; if they are neutralizing, they attenuate the efficacy of treatment
- formation of antibodies against drugs (e.g. compounds acting against TNF) depends also on presence of infection
- **high price**
- **activity and security frequently insufficiently guaranteed**
- **poor biological availability requiring special methods of application**