Large molecules as drugs: artificial antibodies, modified receptors, and pieces of altered nucleic acid as drugs.

A small trip into the realm of biologic drugs

Large molecules = biologics or biological drugs

•officially (WHO) „biological and biotechnological substances“

Basic characteristics of biologics

•typically obtained 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

• due to their hydrophilicity and big M_r they cannot be absorbed from GIT and thus must be applied parenterally

•but all the above conditions don´t have to be necessarily fulfilled for classification of a drug as biologic

Therapeutic monoclonal antibodies

"NORMAL" 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 one 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 non-covalent interactions

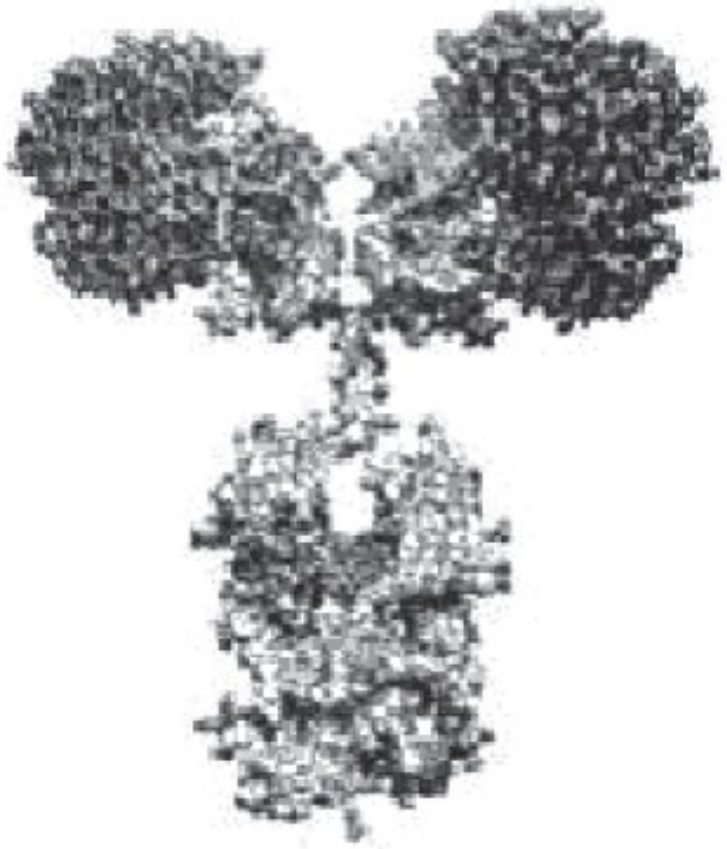
•within the light and heavy chains are domains, which consist of about 110 amino acids of similar polypeptide sequence: constant domains C_H1, C_H2, and C_H3 of the heavy chain

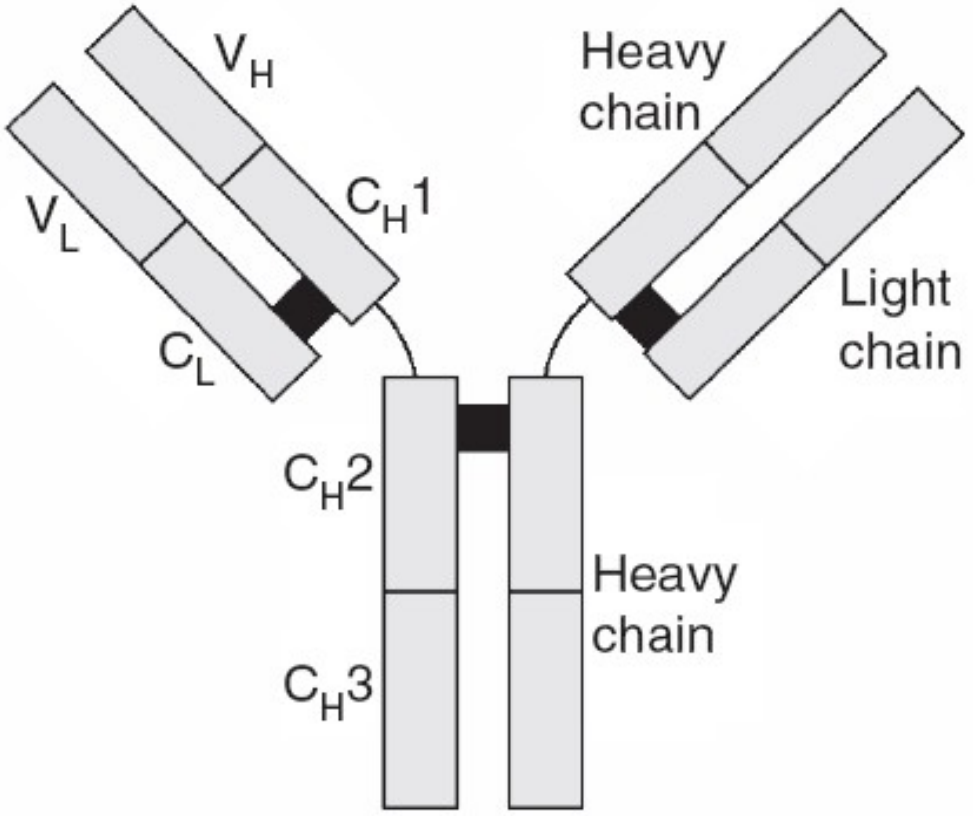
C_L domain of the light chain

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

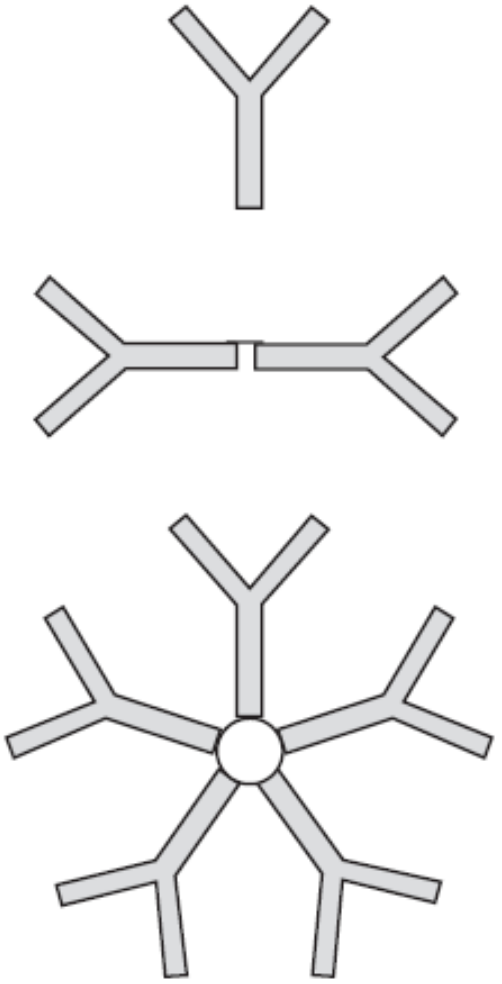
•the variability is confined to particular regions of the variable domain, called the complementarity - determining regions. These regions have the appropriate 3D structure to bind to antigens.

Antibody structure





Typical quartenary structure of immunoglobulins

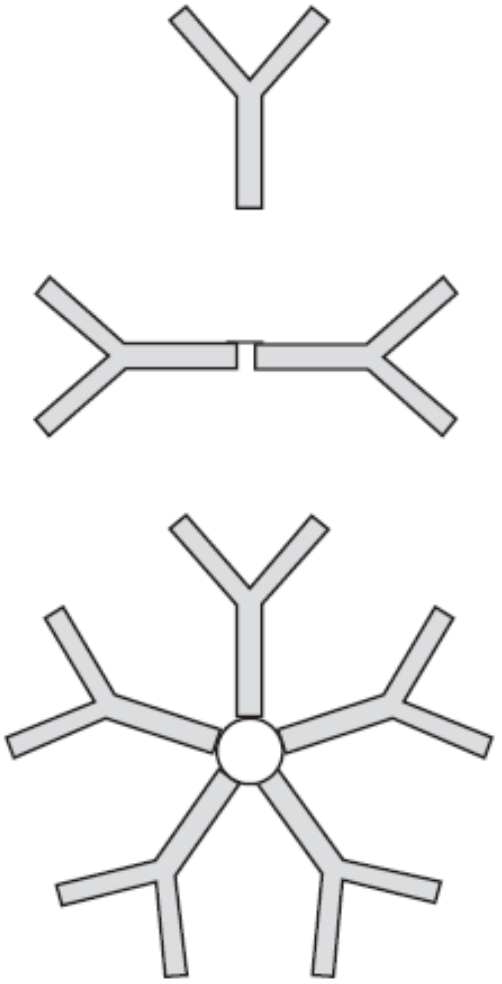


IgG, IgD, IgE
(monomer)

IgA
(dimer)

IgM
(pentamer)

Typical quartenary structure of immunoglobulins



IgG, IgD, IgE
(monomer)

IgA
(dimer)

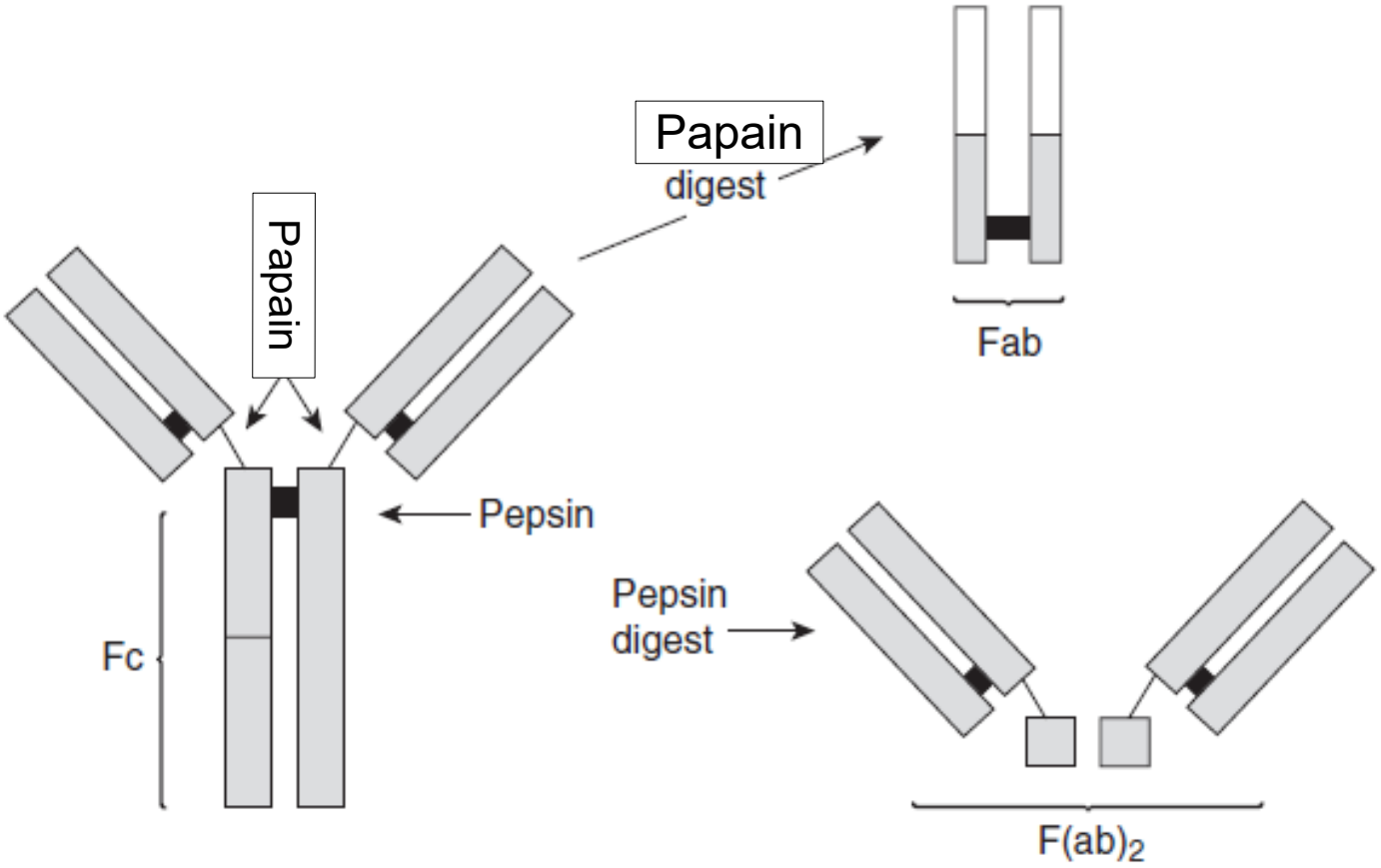
IgM
(pentamer)

Antibody structure fragmentation

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

- Variable Fragment (F_v): The tips of the two Y arms vary greatly from oneantibody to another. They are the regions that bind to epitopes of antigens and bring them to the natural killer cells and macrophages fordestruction.
- Antigen - Binding Fragments (F_{ab}), $F_{ab'}$, and $F_{(ab')\ 2}$: Various parts thatcontain the variable fragment.
- Constant Fragment (F_c): 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 F_c. The F_c 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 European Pharmacopoeia
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.

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

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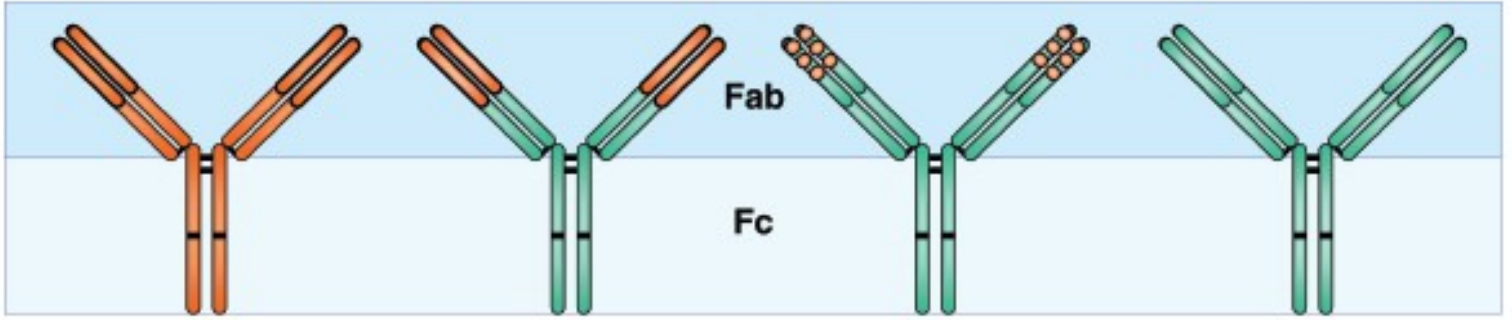
Examples of mabs in therapeutical use woldwide



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

Antincancer drugs

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

•few years ago, falsifications of Avastin ® were catched in U.K.

trastuzumab

Herceptin ® (orig.), Ontruzant ® (biosimilar)

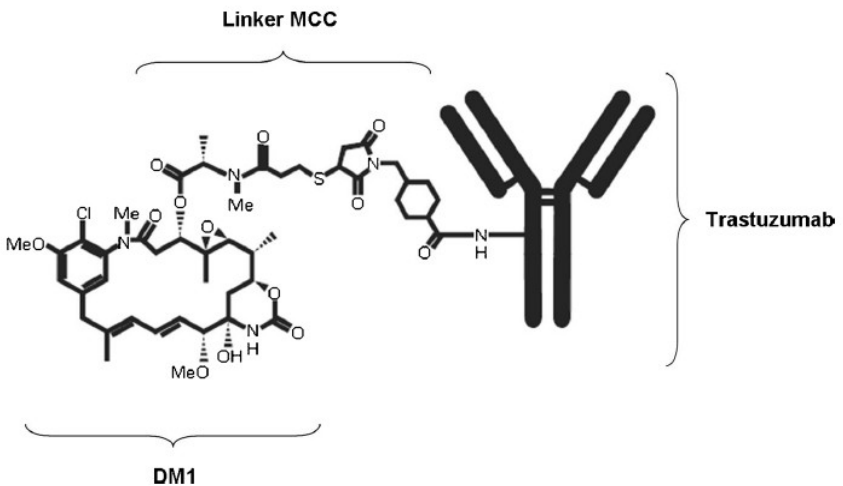
- humanized
- IgG1 κ anti – HER2
- family of receptors for epidermal growth factor includes 4 structurally very similar receptors: Erb/HER (EGFR; HER-1, and ERBB1), human EGFR-2 (HER-2 and
- ERBB2), HER-3, a HER-4, transmembrane glycoproteins containing a domain binding an intracellular ligand and an 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 combined 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.

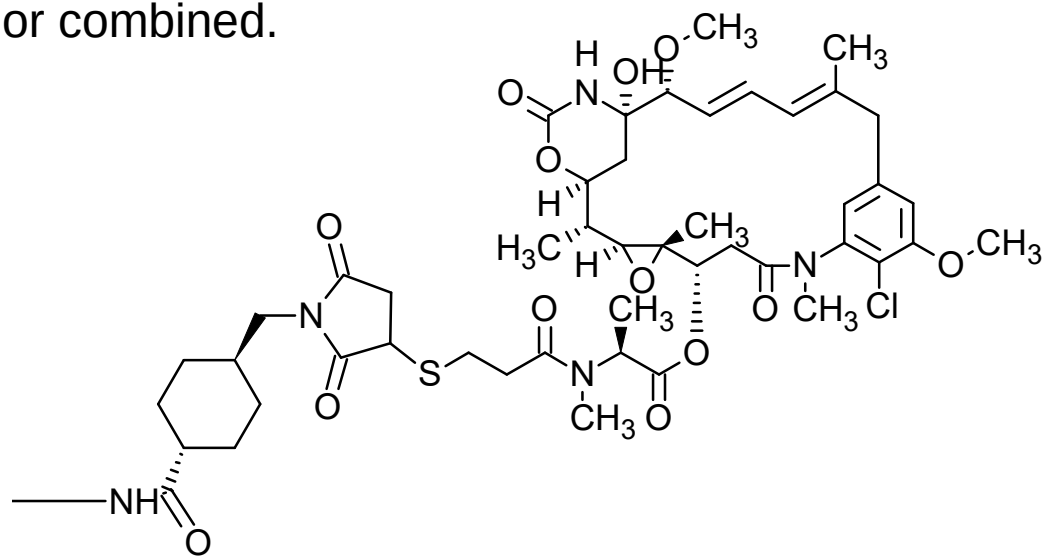
trastuzumab emtansin

Kadcyla ®

- a covalent conjugate of trastuzumab with maytansinoide DM1 – a dysruptor of microtubules assembly
- conjugation through 4-(*N*-maleimidomethyl)cyclohexane-1-carboxyl, linked through a stable thioether bridge into pos. 3 of maleinimide fragment

  - indicated for treatment of adult patients with HER2-positive non-resectable locally developed or methastasizing breast cancer, previously treated with trastuzumab and a taxane, either alone, or combined.





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

Antiviral mabs

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

⇒ 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) ⇒ 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 bamlanivimab 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

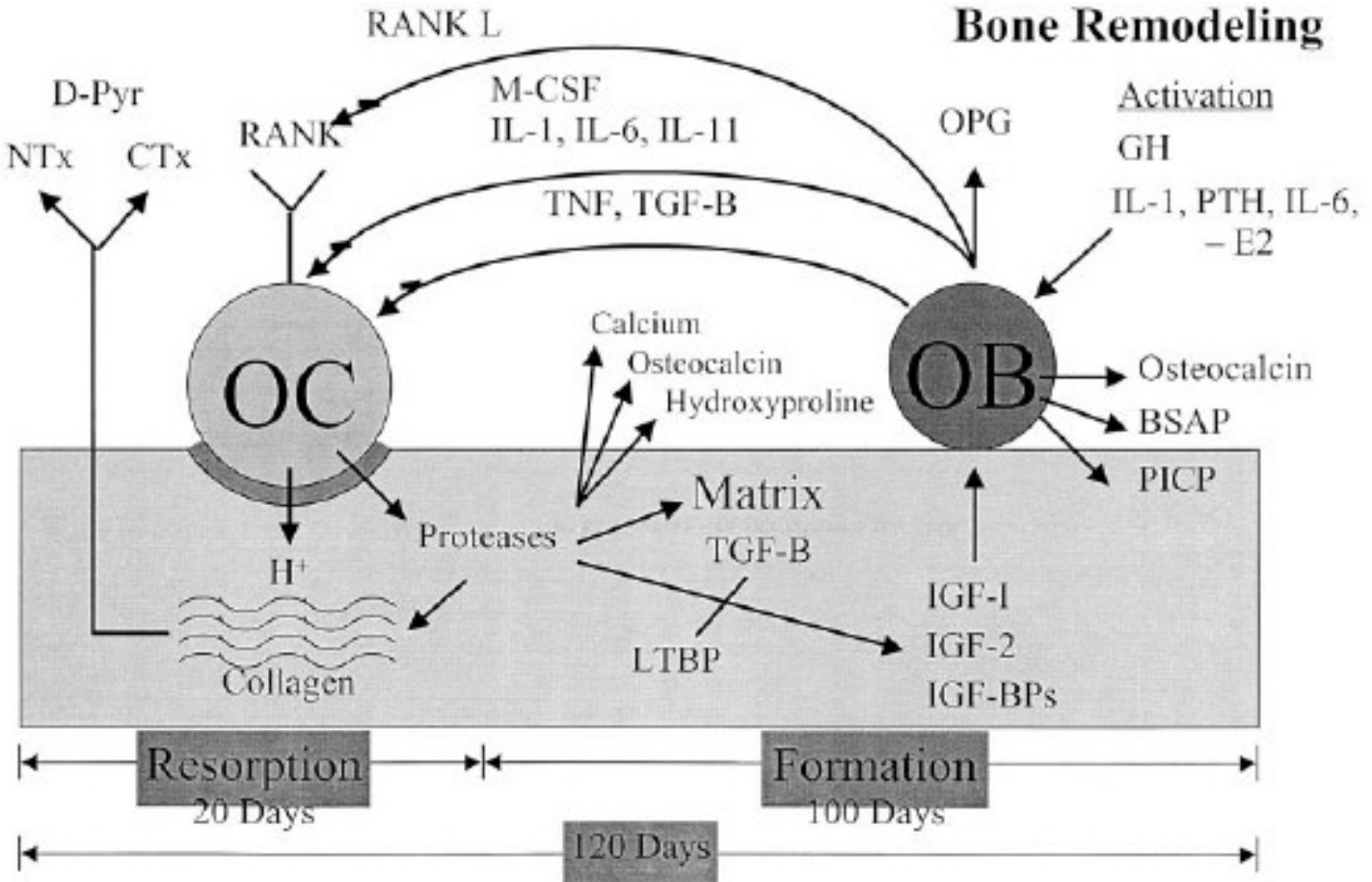
sotrovimab
syn. VIR-7831, GSK4182136

Ig G_1 (438-Leu,444-Ser), anti-(severe acute respiratory syndrome coronavirus 2 spike glycoprotein receptor-binding domain) (human monoclonal VIR-7831 gamma1-chain), disulfide with human monoclonal VIR-7831 kappa-chain, dimer

- 6 ongoing clinical trials mostly in USA

Mabs for osteoporosis

Cyclic bone remodeling



OC = osteoclast; OB = osteoblast; GH = growth hormon; IL = interleukins; E2 = estrogenes; PTH = parathormon; RANK L = osteoprotegerin ligand = ligand of receptor activator of nuclear factor κβ; RANK = receptor for RANK L; M-CSF = macrophages colony stimulating factor etc.

denosumab

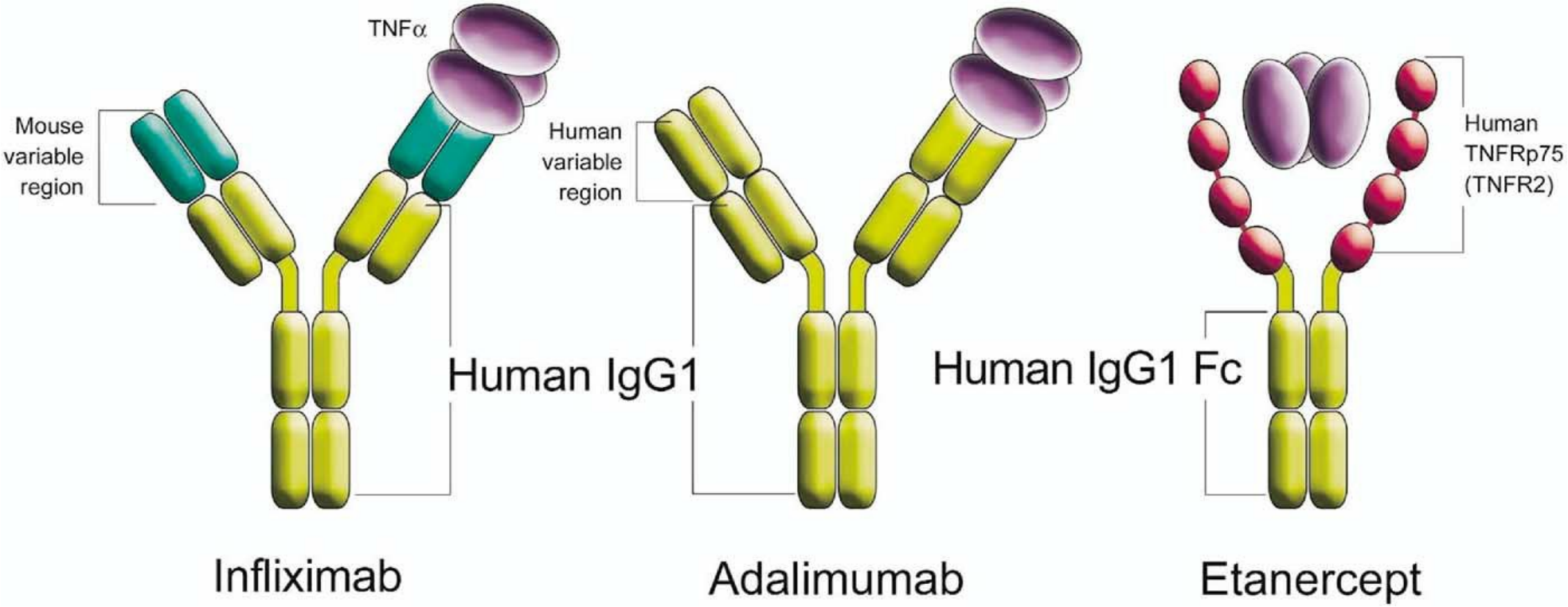
- humanized
- IgG_2
- against RANK L = ligand of receptor activator of nuclear factor kappa beta
- binds specifically to RANK L and blocks its binding to RANK $\Rightarrow$ inhibition of osteoclast formation $\Rightarrow$ decrease of bone resorption
- Prolia ®, Xgeva ®
- indicated in osteoporotic fracture or impossibility of another treatment
- 60 mg *s.c.* / 6 měsíců

Mabs 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 tumour 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. There are two types of glycoprotein drugs blocking TNF – α: anti - TNF - α monoclonal antibodies and modified TNF - α receptors. These drugs 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.

Some TNF-α inhibitors used for longer time



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

Infliximab (Remicade ®) : mouse/human chimera, 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, humanized antibody $F_{ab'}$ 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 F_c domain of human IgG_1

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

Modified receptor molecules used as medicines

Nomenclature of receptor molecules or membrane ligands, native or modified, after WHO

- Common stem **-cept**
  - a preceding infix:
    - **-ba-** for B-cells factor activating receptors
    - **-ber-** for VEGF (vascular endothelial growth factor) receptors
    - **-co-** for complement receptors
    - **-far-** for the subgroup of interferone receptors
    - **-fri-** for *frizzled* receptors family
    - **-ki-** for interleukine receptors
    - **-lefa-** for CD58 receptors (lymphocyte function associated antigen 3, LFA3)
    - **-na-** for interleukin 1 receptors
    - **-ner-** for TNF (tumor necrosis factor) receptors
    - **-ta-** for CTLA4 (cytotoxic T-lymfocyte antigen 4) receptors
    - **-taci-** for transmembráne activator and calcium modulator and cyclophiline ligand interactor
    - **-ter-** for TGF (transformation growth factor) receptors
    - **-vir-** for antiviral receptors

etanercept

- fusion protein of the sequence 1-235 of human p75 TNF receptor with part 236-467 of human IgG_{γ} (= F_c fragment)
- 934 AA
- M_r of aglycone 51 166.8; total cca 150 000
- preparation by a recombinant technology on chinese hamster ovary cell lines
- suluble
- MA: binds to TNF, inhibits its binding to endogenous TNF receptors $\Rightarrow$ pro-inflammatory effect supressed

Enbrel ®, Benepali ®, Erelzi ®, Nepexto ® - 25 or 50 mg, *s.c.* administration, prefilled syringes or pens

- Indicatios (combined with methothrexate, or alone):
  - rheumatoid arthritis
  - polyarticular juvenile arthritis (children over 2 years)
  - psoriatic arthritis
  - ankylosing spondylitis (Bechterev disease)
  - plaque psoriasis (PsO) in patients 4 years or older

inbakicept

syn. ALT-803

- fusion protein of interleukine-15 (IL-15) receptor α-chain (=fragment containing “sushi“ domain) with F_c fragment of human IgG_1, dimer
  - = sequence 1-65 of α-chain of IL-15 receptor + [232 C-terminal residues (66 - 297) + a linker (71-80) + part 81-190 of constant domain 2 of the heavy chain (CH2) + 191 of domain 3 of the heavy chain (CH3)] IgG_1, dimer
- $C_{2980}H_{4624}N_{800}O_{894}S_{28}$ (only aglycon)
- originally proposed as an anticancer drug
- *i.v., s.c.*, *i.p.* administration
- clinical studies
  - decrease of persistence of HIV virus in lymphatic nodes (phase 2)
  - pharmocokinetics after *s.c.* administration (phase 1)
  - preparation of NK cells from a donor for usage in treatment of acute myeloid leukemia (phase 2)
  - relapsing or multiple refractory myeloma (phase 1)

aflibercept

Eylea ® *intravitreal injection; pre-filled syringe*

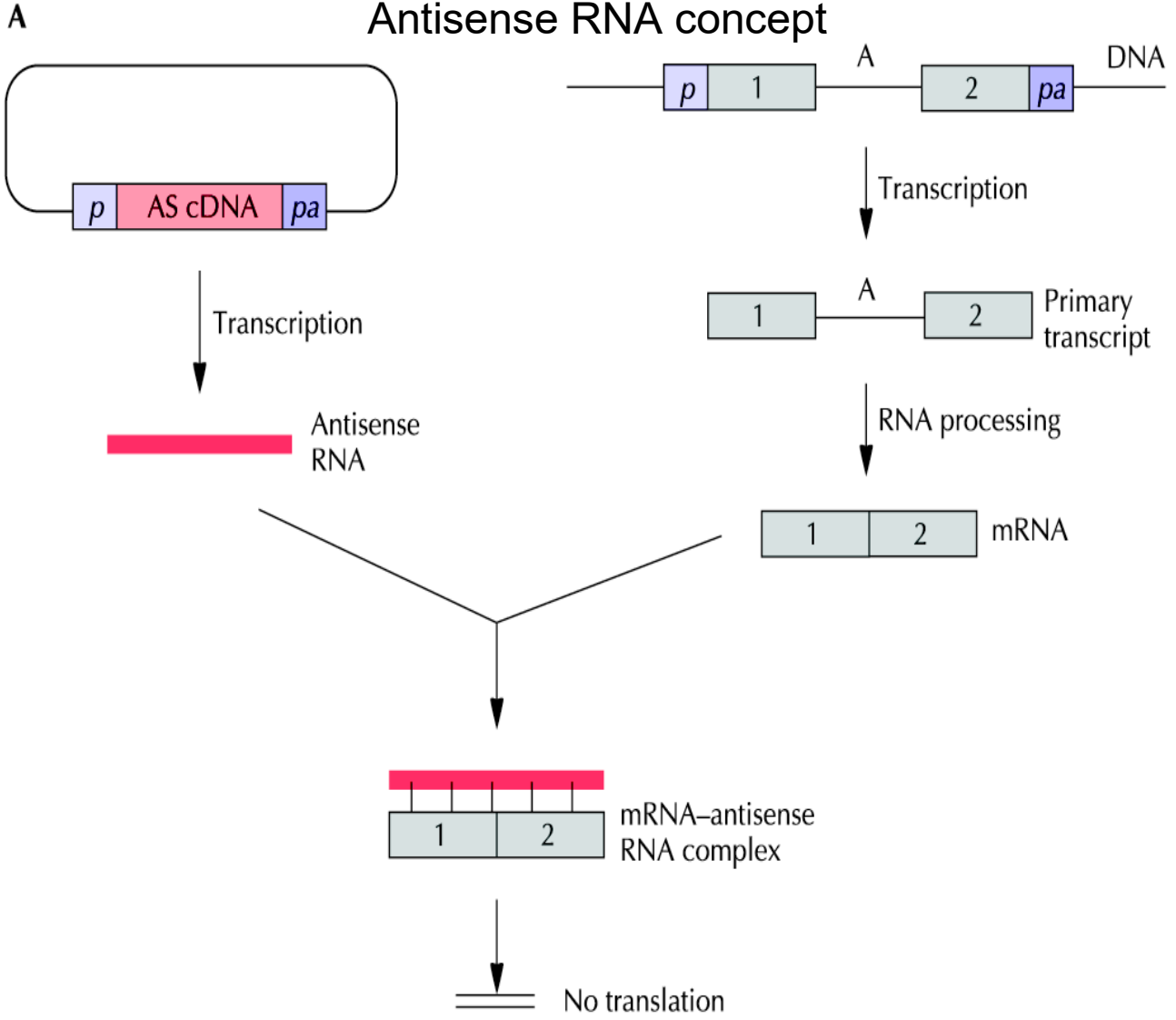
- antiangiogenic activity
- a heterodimeric fusion protein consisting of portions of human VEGF (Vascular Endothelial Growth Factor) receptors 1 and 2 extracellular domains fused to the F_c portion of human IgG_1
- produced in Chinese hamster ovary (CHO) K1 cells by recombinant DNA technology
- originally proposed as an anti-neoplastic
- acts as a soluble decoy receptor that binds VEGF-A and PlGF with higher affinity than their natural receptors, and thereby can inhibit the binding and activation of these cognate VEGF receptors.

- Indications:
  - neovascular (wet) age-related macular degeneration (AMD)
  - visual impairment
    - due to macular oedema secondary to retinal vein occlusion (branch RVO or central RVO)
    - due to diabetic macular oedema (DME)
    - due to myopic choroidal neovascularisation (myopic CNV)

Antisense oligonucleotides

= short sequences of a modified single strand nucleic acid complementary to parts of damaged DNA sequences

Antisense RNA concept



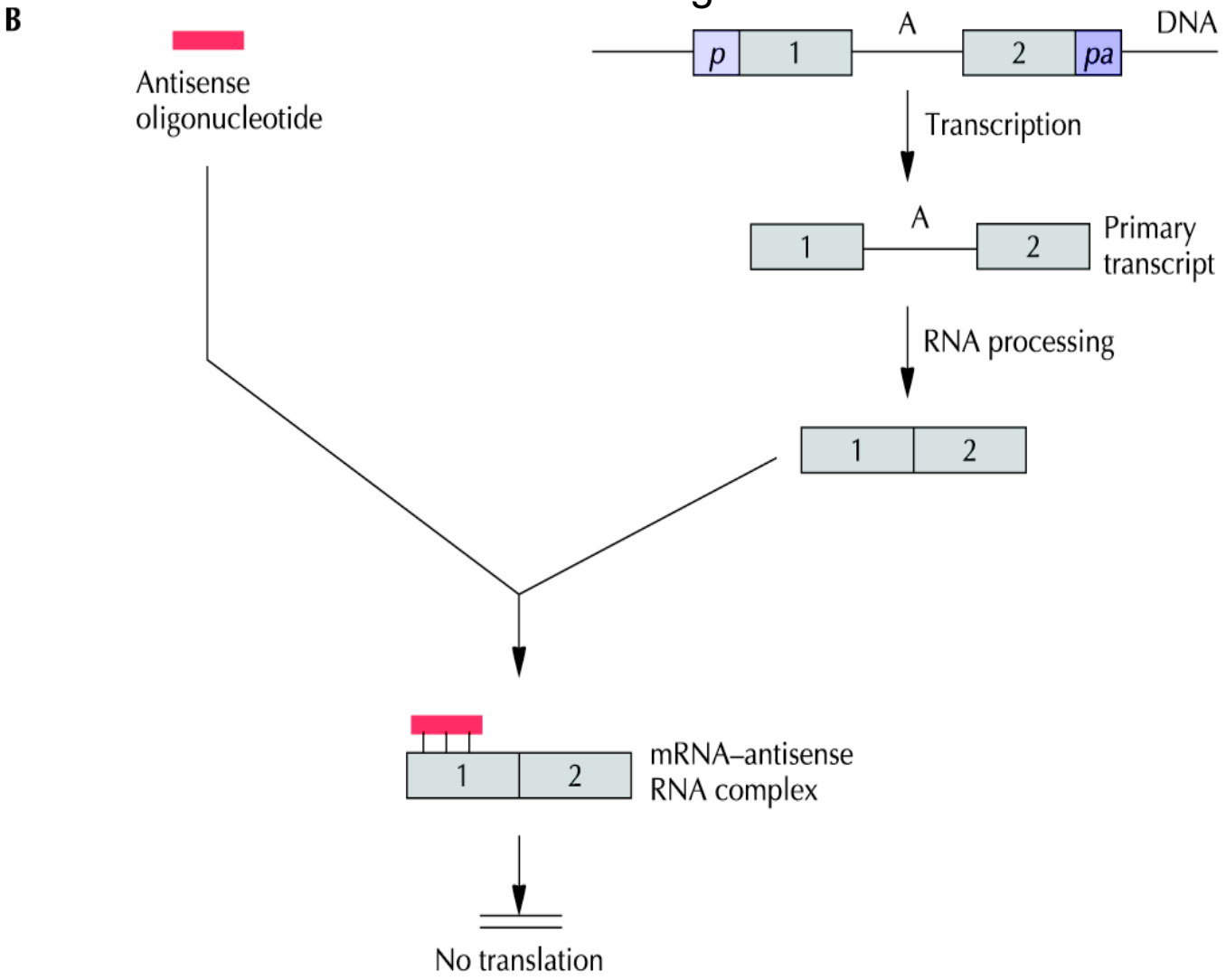
Why Antisense Oligonucleotides?

- Large antisense RNA is readily subjected to degradation
- Human cells specifically target dsRNA for turnover by nucleases

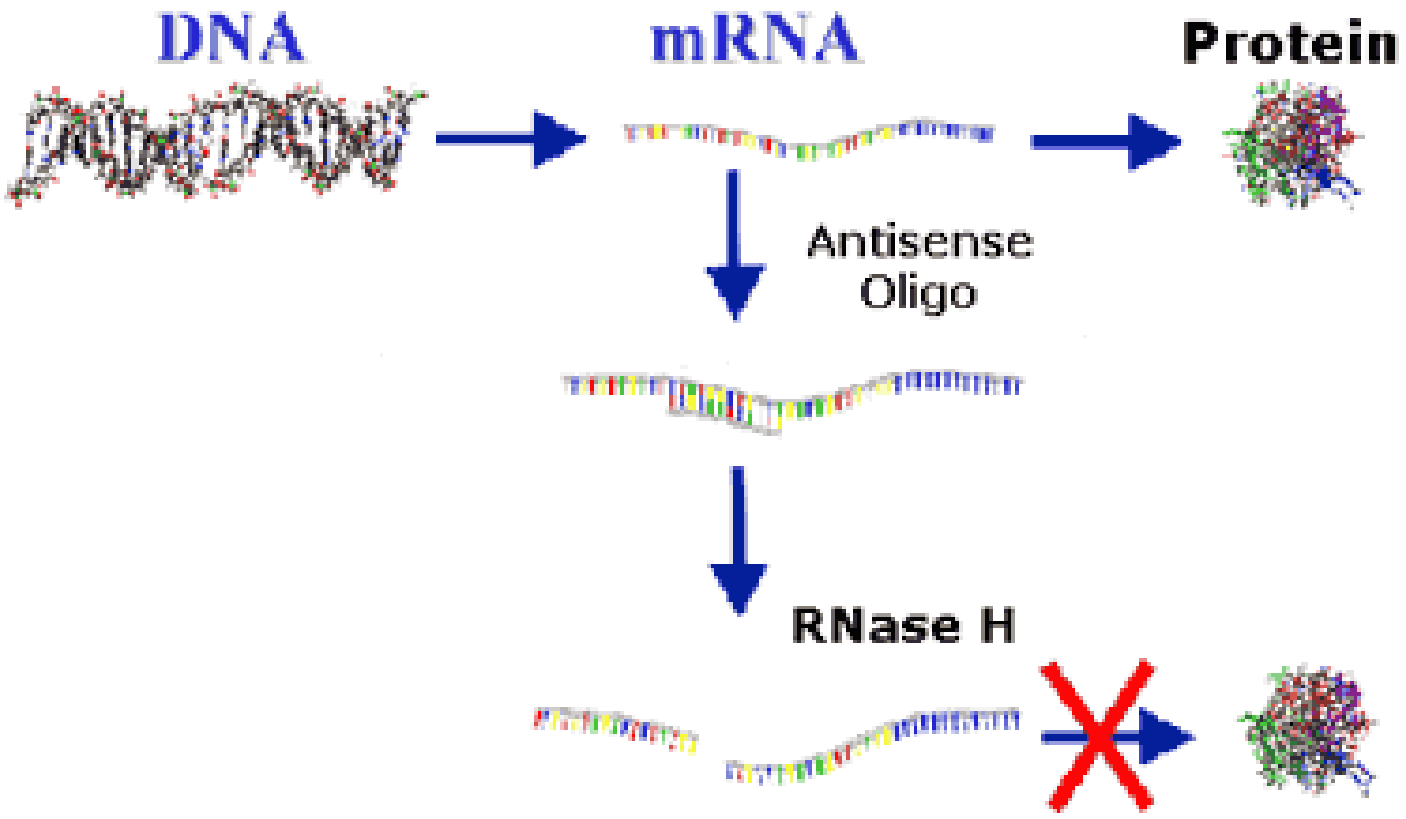
- DNA oligonucleotides more stable, more readily delivered to target cells

- Directed to 5’ or 3’ ends of mRNAs, intron-exon boundaries, naturally ds regions

Antisense Oligonucleotides

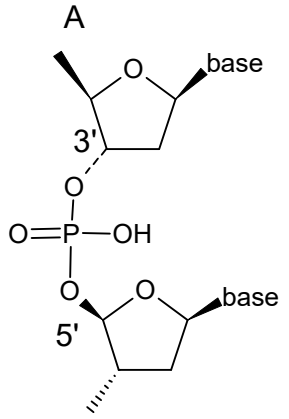


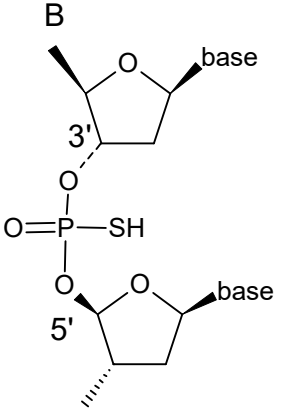
Antisense Oligonucleotides - „mode of action“

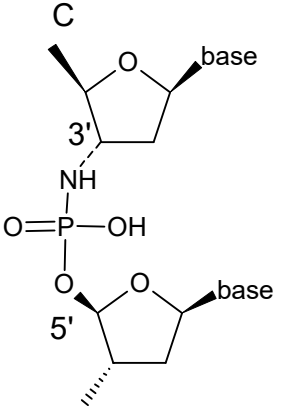


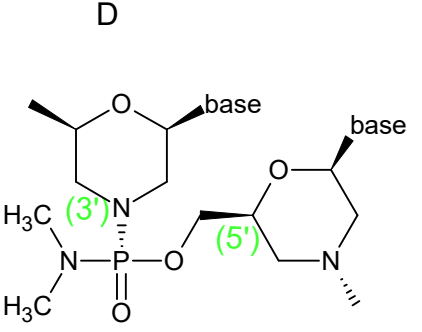
Optimizing Antisense Oligos

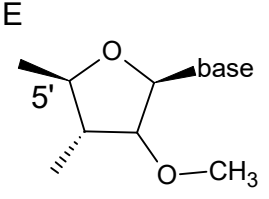
- Natural oligos (A) susceptible to cellular nucleases
- Alterations improve nuclease resistance
- Replacement of free oxygen with sulfur in phosphodiester bond (B) particularly effective
- Replacement of deoxyribose with morpholine ring together with substitution of hydroxyl group in phosphate moiety (D) with dimethylamine led to clinically used compounds (eteplirsen)

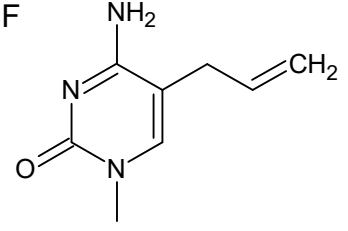












Morpholine oligos

eteplirsen, AVI-4658, Exondys 51 ®

•treatment of Duchenne muscular dystrophy (DMD)

•DMD: a fatal muscle degenerative disorder, caused by misreading and bad translation of DMD (dystrophin) gene to dystrophin peptide due to mutations of this gene

•1 per 3500 newborn boys is affected

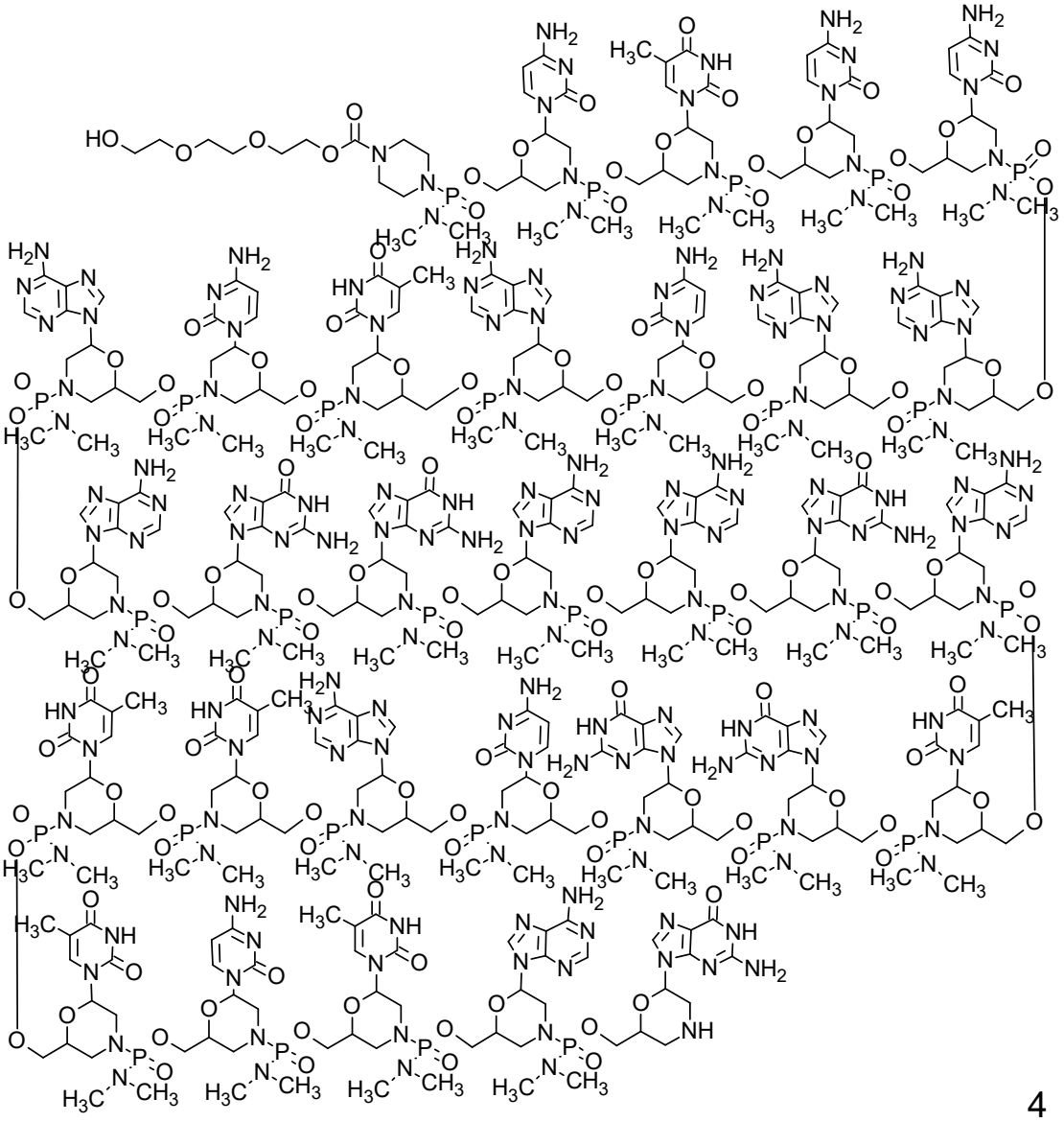
•sequence CTCCAACATCAAGGAAGATGGCATTTCTAG; 30 bases; M_r = 10369.83

•targeted to 51. exone in dystrophine mRNA

•enables partial restoration of reading and translation of DMD gene by „patching“ of its bad splicing $\Rightarrow$ production of truncated, but functional chain of dystrophine

•applied locally by i.m. injection in clinical trials
•therapeutically 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 available

Complete structure of eteplirsen



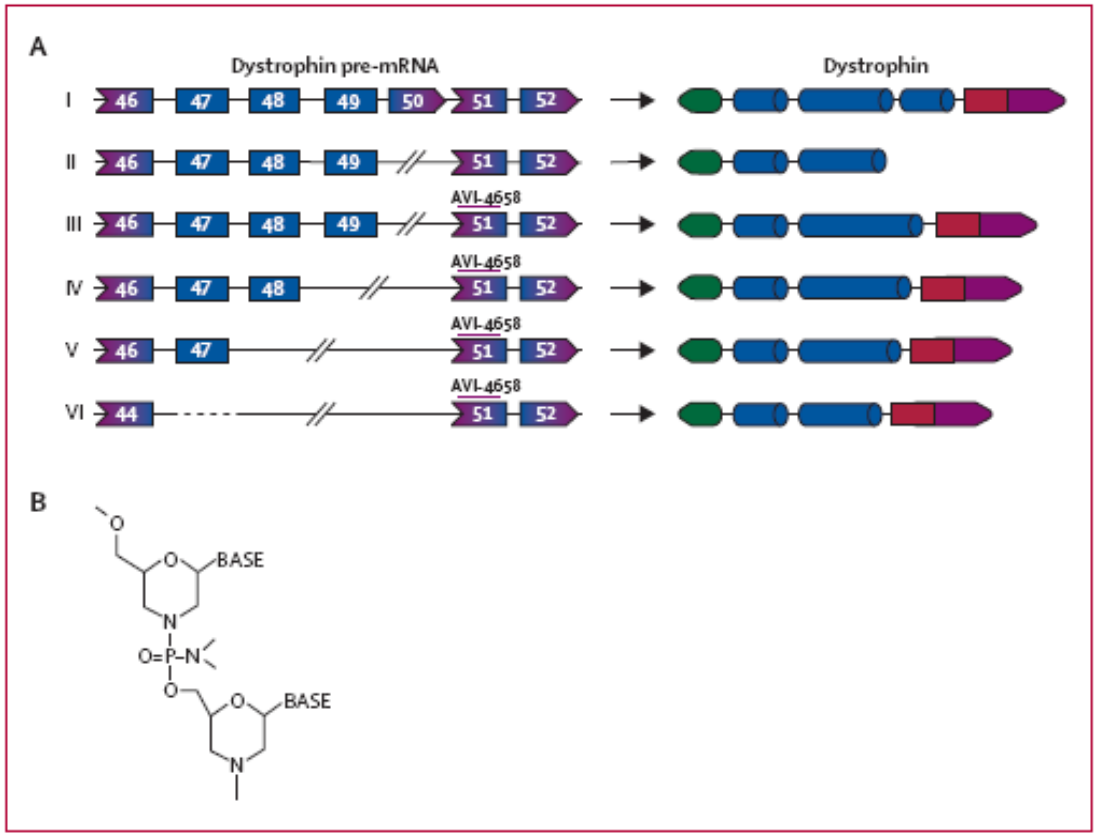


Figure 1: **Deletions and predicted results of exon skipping in the patients who were studied**
(A) Pre-mRNA transcripts and dystrophin protein products from full length *DMD*, in patients with Duchenne muscular dystrophy, and predicted protein sequences after exon skipping. (I) The normal dystrophin 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.

Compounds in clinical trials: antincancer drugs

Oncogene Bcl-2 antagonist

•Bcl-2: antiapoptic protein; its dominancy over structuraly related proapoptic Bax presents a bad response of tumors to common anticancer therapies and bad prognosis

oblimersen, G3139, BP-1002, augmerosen, Genasense®

•deoxyribonucleotide, 18 nucleotides, phosphorthioate, heptadecasodium salt

•T-C-T-C-C-C-A-G-C-G-T-G-C-G-C-C-A-T

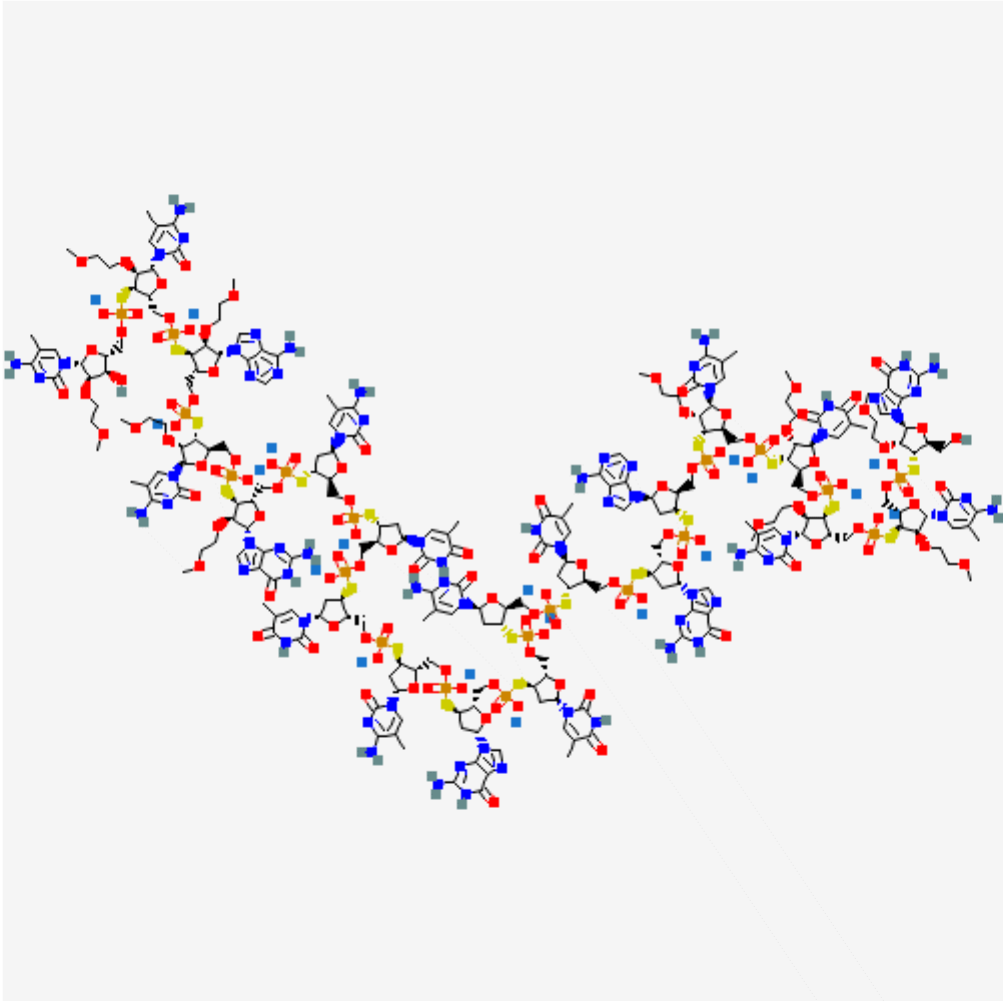
•complementary to first six codones of human Bcl-2 mRNA

•administered by podání *i.v.* infusion

•passed 45 1st – 3rd phases clinical trials agaist various types of cancer; efficient; rel. low toxicity

•currently one ongoing phase 1 study which evaluates the safety and tolerability of escalating doses of BP1002 (Liposomal Bcl-2 Antisense Oligodeoxynucleotide) in patients with refractory/relapsed acute myeloid leukemie (AML) . The study is designed to assess the safety profile, biologically effective doses, pharmacokinetics, pharmacodynamics, and potential anti-leukemic effects of BP1002 as single agent (dose escalation phase) followed by assessing BP1002 in combination with decitabine (dose expansion phase).

Compounds that were recently used: antihyperlipidemics
mipomersen sodium, Kynamro®



- phosphorthioate; a methoxyethyloxy substitution at position 2´of each ribose unit
- 20 bases
- complementary to **human apolipoprotein B-100 (apoB-100) m RNA**
  - subsequently reduces translation of ApoB-100 protein, the major apolipoprotein of very low-density lipoprotein, intermediate-density lipoprotein and low-density lipoprotein (LDL).
- approved in USA 2013 – 2019(?)
- (EMA refused approval in EU in May 2013)
- AE: liver steatosis (increase in liver fat ≥ 5 % in 60 % of patients, increase of aminotranserases)
- the recommended dose of KYNAMRO was 200 mg once weekly as a subcutaneous injection.