Biological and gene therapy

Attila Megyeri

16.11.2018

Biopharmaceuticals, gene therapy
(basic concepts)

- proteins
  - „engineered” proteins
  - antibodies
- nucleic acids
- cells / tissues

not produced by chemical synth. !

- biological therapy
  - in vitro production → in vivo use
- gene therapy
  - reprogramming of living somatic cells
  - in vivo or ex vivo

Biopharmaceuticals

(Biological products)

- large complex molecules
  - proteins
    - **“engineered”/recombinant proteins**
      - e.g. antibodies, protein hormones
    - vaccines
    - blood, blood components
    - allergenics
  - nucleic acids
    - antisense oligonucleotides
    - siRNA
    - *gene therapy*
- cells, tissues
  - somatic

Biopharmaceuticals
(Biological products)

- **How they are produced?**
  - **isolation/biotechnology = cellular production**
    - mixtures
    - chemical structure is not exactly defined
    - susceptible to microbial contamination
  - *this is in contrast with chemical synthesis*
    - purified
    - chemical structure is well known
    - no microbial contamination
- **in vitro production ↔ in vivo use**

Proteins

- e.g. insulin / growth hormone
  - originally
    - source: animal / human
    - problems: low yield / immune response / infections
  - currently
    - source: transfected expression system
    - advantage: good yield
    - problems: endotoxin / glycosylation / cost

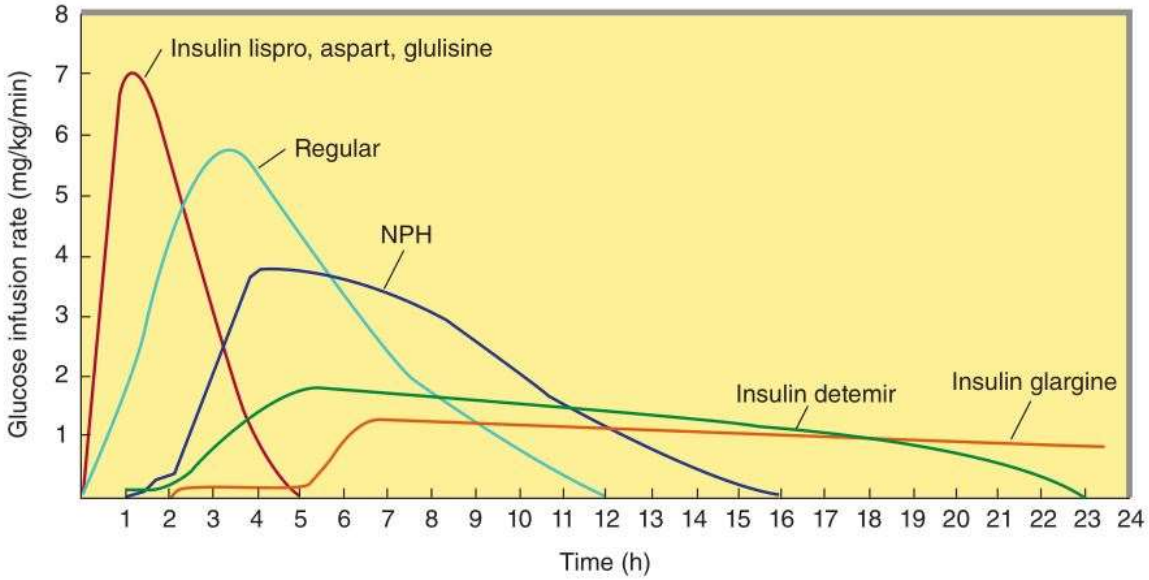
Proteins

- exact copies of proteins (1st gen)
- modified proteins (2nd gen)
  - improved pharmacokinetics
    - pegylation / insulin
  - fusion proteins – novel function
    - etanercept / immunotoxins
  - reduced immunogenicity (humanization)
- proteins designed from scratch (3rd gen)
  - future

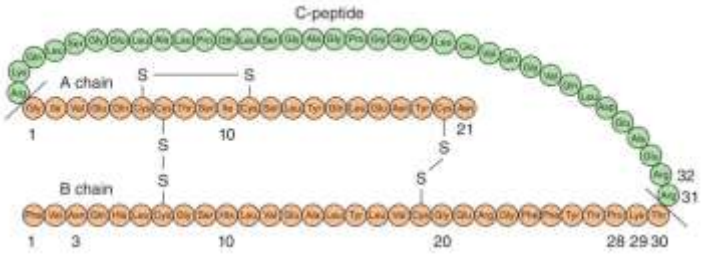
Examples of biologics

- hormones
  - insulin
  - growth hormone
  - pegvisomant
    - GH receptor antagonist – to treat acromegaly
    - polyethylene glycol (PEG) derivative of a mutant GH
- growth factors / cytokines
  - erythropoietin
  - granulocyte colony stimulating factor (G-CSF, filgrastim)
  - interferons (IF-α, IF-β, IF-γ)
  - interleukins (e.g. IL-2)
- antibodies

Insulin preparations

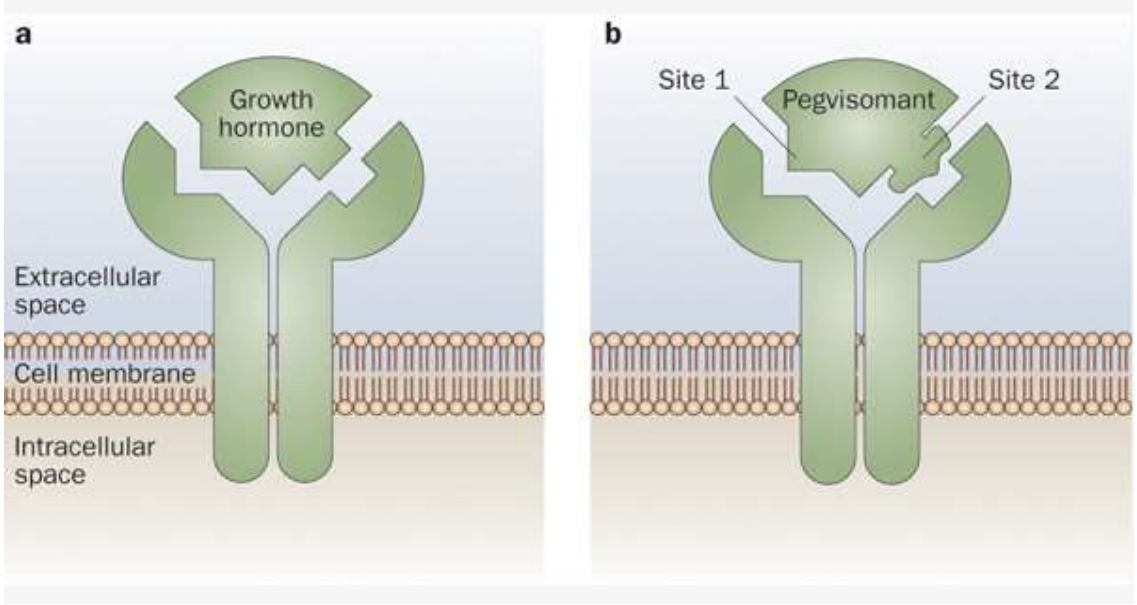


- rapid acting
  - lispro – B28 Pro ↔ B29 Lys
  - aspart – B28 Pro → Asp
  - glulisine – B3 Asn → Lys + B29 Lys → Glu
- long-acting
  - detemir – terminal Thr ↔ myristic acid
  - glargine – add 2 Arg to B carboxy + A21 Asn → Gly
  - degludec – similar to detemir (Thr ↔ hexadecanedioic acid)



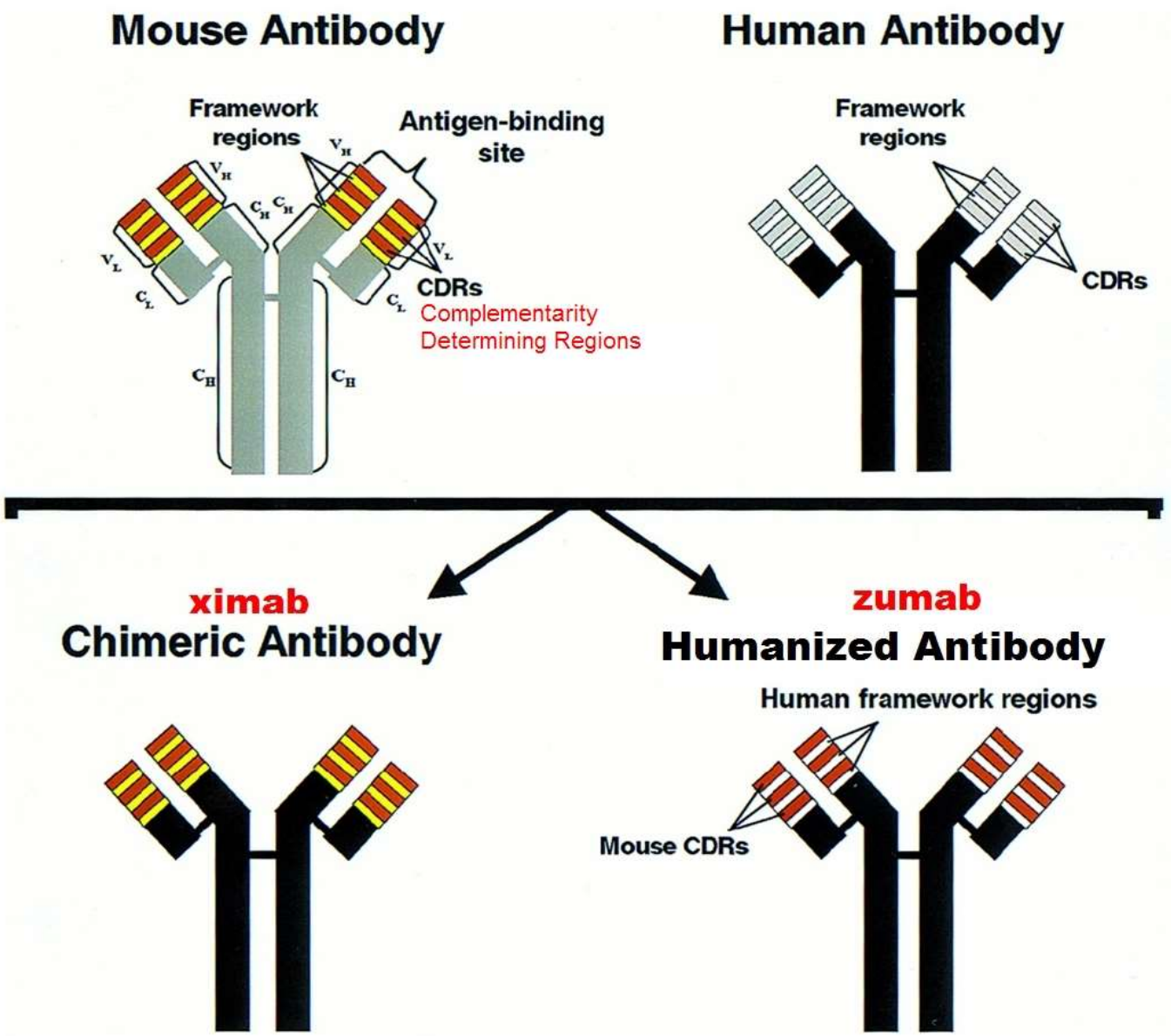
Pegvisomant

- **PEG** derivative of a mutant GH
- used/effective in acromegaly
- increases circulating GH conc.



Antibodies

- polyclonal antibodies
- monoclonal antibodies – hybridoma
  - murine
  - chimeric
  - humanized
  - human

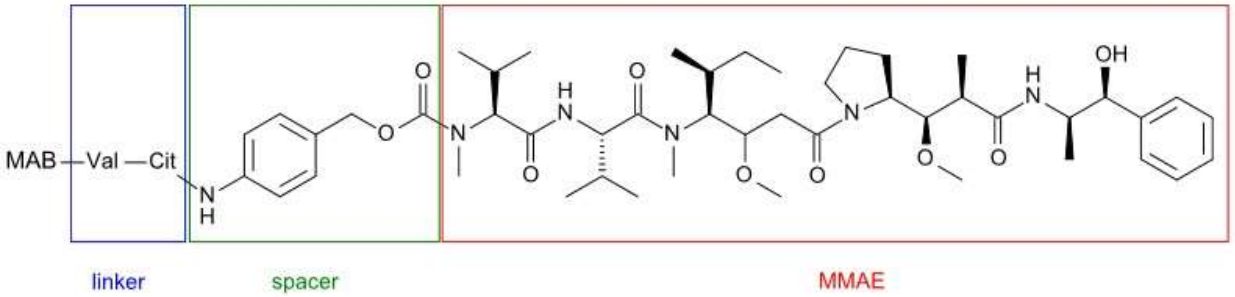

Mouse Antibody
Framework regions
Antigen-binding site
CDRs
Complementarity Determining Regions
Human Antibody
Framework regions
CDRs
ximab
Chimeric Antibody
zumab
Humanized Antibody
Human framework regions
Mouse CDRs

Advantages of humanization of antibodies

- half-life is longer
- reduced immunogenicity
- more effective activation of human effector mechanisms
  - ADCC (Antibody Dependent Cellular Cytotoxicity)
  - complement activation

Immunotoxin

- **brentuximab vedotin** (target: CD30)
  - anti-CD30 chimeric IgG1 + MMAE
    - binding → internalization → release of MMAE → blockade of tubulin polymerisation
  - indication (**but only after failure of other therapies**)
    - anaplastic large cell lymphoma
    - Hodgkin's disease
  - risk of progressive multifocal leukoencephalopathy (PML)

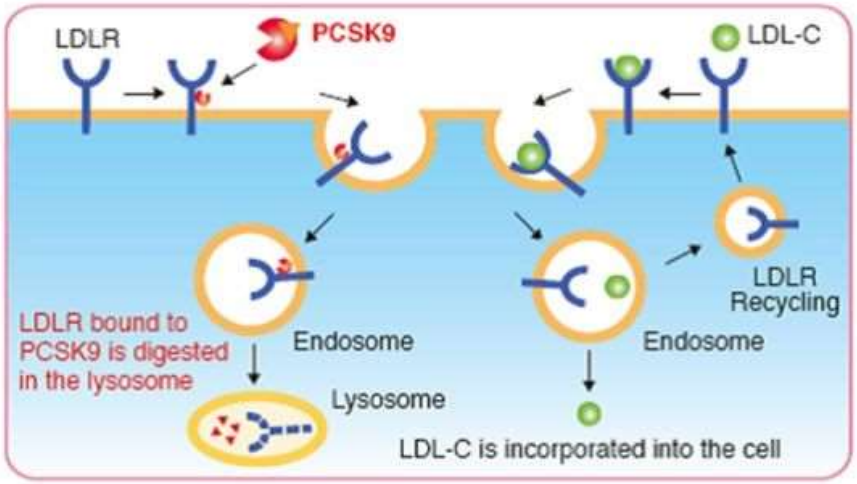


Biologic therapy for hyperlipidemia

PCSK9 inhibitors

(**p**roprotein **c**onvertase **s**ubtilisin/**k**exin type **9**)

PCSK9 protease → binds to LDL rec. → ↑ degradation → ↑ **LDL**



Expensive – 14100 USD/year (2015)
"On October 26, 2018 Amgen announced a 60% cut in price (5850 USD/year)"

Biologic therapy for hyperlipidemia

PCSK9 inhibitors

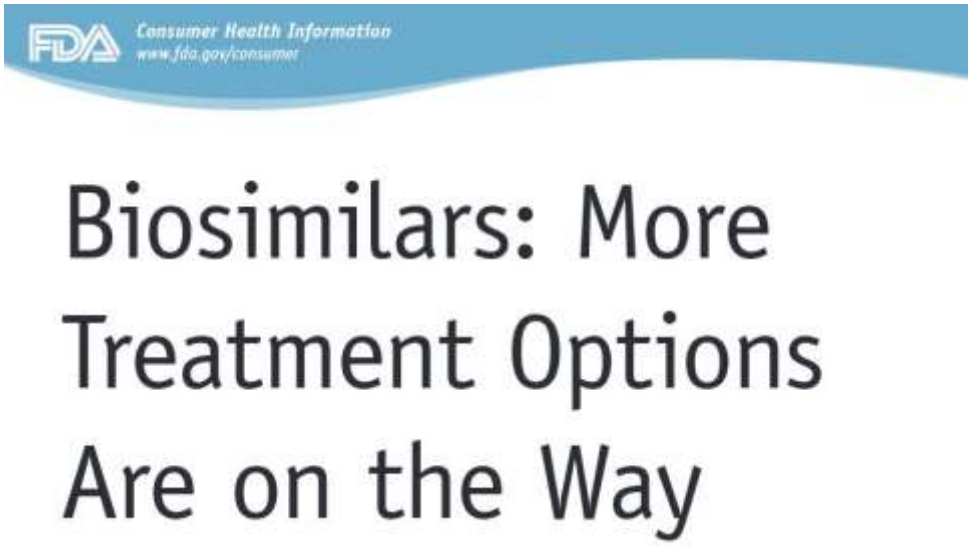
(**p**roprotein **c**onvertase **s**ubtilisin/**k**exin type **9**)

- PCSK9
  - protease → binds to LDL rec. → ↑ degradation → **↑ LDL**
- **alirocumab / evolocumab**
  - **fully human monoclonal antibodies against PCSK9**
- **sc.** biweekly → up to 70% LDL ↓ + triglycerides, apo B-100 and Lp(a) ↓
- **adjunctive** to diet and maximally tolerated statin and/or ezetimibe
- indications
  - **familial hypercholesterolemia**
  - est. atherosclerotic cardiovascular disease requiring additional LDL lowering
- adverse effects
  - **inj. site reactions**
  - ↑ infection risk (upper resp. / urinary)
  - hypersensitivity (rare)
  - neurocognitive effects ?
  - no ↑ risk of myopathy

Drug development

- manufacturing
  - quality assurance
  - aseptic technology
- preclinical / clinical development
  - biosimilarity (not generic)
    - higher sensitivity to changes in manufacturing processes
  - safety
    - high specificity – see TGN1412

Biosimilars



- Zarxio: First US FDA approved biosimilar
  – 06.03.2015 (but EU approval: 06.02.2009)
- Neupogen (filgrastim) → Zarxio

Biological therapy ≠ targeted therapy

- anticancer molecularly targeted therapy
  - non-biologicals
    - tyrosine kinase inhibitors
      - e.g. imatinib, dasatinib, nilotinib (BCR-ABL kinase, CML)
    - epidermal growth factor receptor inhibitors
      - e.g. lapatinib (HER2+ breast cancer)
      - e.g. erlotinib (metastatic non–small cell lung cancer)
  - biologicals
    - epidermal growth factor receptor inhibitors
      - e.g. trastuzumab (HER2+ breast cancer)
      - e.g. cetuximab (EGFR+ metastatic colorectal cancer)
- DMARDs
  - non-biologicals
    - e.g. methotrexate, hydroxychloroquine, leflunomide
  - biologicals
    - e.g. infliximab, adalimumab, etanercept

Gene therapy

- genetic modification of **somatic** cells
  - transfer of recombinant nucleic acid into target cells
  - in vivo ↔ ex vivo
- goals
  - prevent
  - alleviate
  - cure
- **potential** use
  - cure of monogenic diseases
    - e.g. cystic fibrosis, haemoglobinopathies
  - ameliorate diseases with or without a genetic background
    - e.g. malignant, neurodegenerative
- major problems / considerations
  - delivery (capacity-efficiency-selectivity) (i.e. PK)
  - safety
  - clinical efficacy
  - long term predictability (persistence)

Requirements of gene delivery systems

- capacity of the system
  - how much DNA it can carry
- transfection efficiency
  - ability to enter and become utilized by cells
- lifetime of the transfected material
  - determined by the lifetime of the targeted cells
- safety
  - especially in the case of viral delivery systems

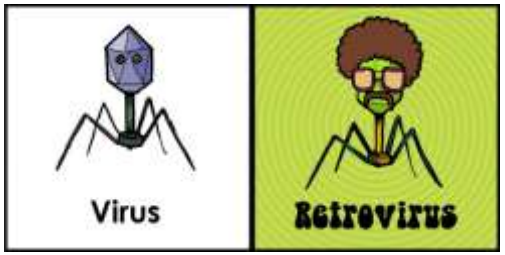
Gene delivery

- in vivo
  - vector is injected into the patient
  - iv. (targeting) or directly into the target tissue
- ex vivo
  - remove cells from the patient
  - treat them with the vector
  - inject the genetically altered cells back

ideal vector: safe, efficient, selective, results in persistent expression

Gene delivery

- viral vectors (modified 'replication defective‘)
  - retroviruses
    - incorporated randomly into host DNA
    - only infect dividing cells
    - persistence (if into stem cells)
    - little specificity (ex vivo use)



  - adenovirus
    - high transgene expression
    - not inserted into the host genome > do not replicate > only temporary effect
    - low dose inefficient while high dose elicit immune response
  - other viruses
    - adeno associated virus / herpesvirus / disabled HIV

Gene delivery

- non-viral vectors
  - liposomes
    - positively charged lipids ('lipoplexes')
    - improved delivery into the cell nucleus
    - much less efficient than viruses
  - microspheres
    - made from polyanhydride co-polymers of fumaric and sebacic acids
    - loaded with plasmid DNA
    - possibility of oral gene therapy
  - plasmid DNA
    - much less efficient / cannot be targeted
    - no risk of viral replication and is not usually immunogenic

Gene expression control

- control the activity of gene
  - e.g. hemoglobinopathies - appropriate balance of normal α- and β-globin chain synthesis
- inducible expression system
  - e.g. doxycycline-inducible promoter

Safety

- general
  - related to the nature of vectors
- specific
  - e.g. polycythaemia from overexpression of erythropoietin
- viral vectors
  - acquire virulence
  - immunogenic viral proteins
  - elicit inflammatory response
  - damage host genome and interfere with the cell cycle > provoke malignancy

Example for problem of persistence

- **cystic fibrosis**
  - autosomal recessive
  - airway epithelium malfunction
  - missing membrane Cl^- transporter
    - cystic fibrosis transport regulator (CFTR)
- continuous replacement of epithelial cells
  - periodic need for treatment
    - unless the gene is inserted into progenitor/stem cells

Gene therapy in Parkinson's disease

- three completed **Phase 1** trials in the US
  - vector: adeno associated virus type 2
  - genes
    - glutamic acid decarboxylase – GABA synthesis ↑ into subthalamic nucleus
    - aromatic acid decarboxylase (AADC) – levodopa → dopamine ↑ in putamen
    - neurturin – neurotrophic factor, may ↑ survival of dopaminergic neurons
  - results: safe and **possibly effective**
  - Phase 2 trials are under way

Suicide gene therapy for GvHD

- background
  - in high-risk hematological diseases
    - allo-SCT from HLA-matched donor is potentially curative
  - antileukemic efficacy is determined by
    - conditioning regimen + alloreactivity
- problem
  - alloreactivity → graft-versus-host-disease (GvHD)
- solution
  - suicide gene → donor T cells ex vivo
    - thymidine kinase gene / caspase-9
  - later posttreatment to eliminate T cells
    - ganciclovir / AP1903 (dimerizing drug)
- result
  - **promise for control GvHD**