MUNI
MED

# Biological treatment - principles, technology, examples

MUDr. Alena Máchalová, Ph.D., Department of Pharmacology

# Biological drug

– „Biodrugs, biologics, targeted treatment “

- recombinant proteins, peptides, antibodies, hormones

*substances derived from blood / plasma and recombination variants*

– „Biologicals, Biopharmacy, Biopharmaceuticals“

- recombinant proteins, peptides, antibodies, hormones

+ Stem cells, xenotransplantation, gene and antisense therapy

MUNI MED

# Targeted effects of biologicals

A specific effect of biologics is targeted - a specific target structure, antigen, enzyme, signal path (e.g. tumor cells).

Biologics are able to identify the damaged cells for destruction by the immune system.

Can prevent the growth and proliferation of cells that cause disease.

Can deliver drug directly to the **target** which increases the effectiveness of treatment.

MUNI
MED

# Traditional/Classical vs. Biological drug

| Traditional/Classical | Biological drug |
|---|---|
| Small molecule <1kDa, different from endogenous substances | Large, complex molecules, commonly proteins> 50 kDa, similar or identical to endogenous |
| Produced by chemical synthesis / isolation from plants | Manufactured using living organisms / cells<br>- risk contamination<br>- own "inherited„ activity |
| Less critical steps in the synthesis, easier handling and formulation | Complex heterogeneous structure matrix from which was drug isolated |
| Very well characterized | More difficult to characterize (3D conformation) |
| Molecular mechanism of action<br>usually better described, linear dose-response relationship, affects the whole body | Mechanism of action is complex, sometimes not fully understood, targeted action |
| Mostly non-immunogenic | Usually immunogenic |
| Usually with pharmacokinetic interactions at P450 | Mostly without interaction at P450 |

MUNI MED

# Research and development of Biological drugs

The development of biologics is 10 - 15 years, costs 1.5 billion USD

Biologics are produced by the  genetically modified host cells (bacterial, yeast, mammalian and plant) into which was inserted the genetic information stored in DNA.

The first drug produced by biotechnological procedure was insulin (in 1978, registering 1982).

The discovery of biotechnological production of pharmaceuticals, respectively monoclonal antibodies, was in 1984 awarded the Nobel Prize.

# Examples of Biological drugs

1) Imunomodulating biologics

MAb (Infliximab) and fusion proteins (etanercept), IFN

2) Hormones – insulin, GH

3) Vaccines – e.g. HVB, HPV

4) Growth factors – erytropoetin, trombopoetin, CSF

5) Enzymes for the treatment of hereditary diseases (monogenic) (e.g. Imiglucerase for the treatment of Gaucher disease)

6) Biologics influencing homeostasis- f. VII, F VIII, F IX, other inh. Of coaglation or activators of fibrinolysis

7) Gene therapy (e.g.. Alipogen tiparvovek – LPL gene)

# Advantages for the patients

- better efficiency vs. "Classical" drugs
- biologicals are used under the supervision of experts in specialized centers.
- targeted, personalized treatment, which is always personalized
- the patient undergoes a more detailed examination before medication
- better understanding of the basic properties of the drug and its effects
- better solution of possible ADRs, their early detection

# Risks and disadvantages of biological drugs in general

- carcinogenicity
- allergenic potential
- contaminants from the source cells
- stabilizing additives (cryopreservation stabilizers)
- sterility
- stability, variability of drugs (biotechnology products)

# Production of biological drugs

Historically isolation from natural sources:

- insulin from the pancreas of cattle, pigs (recombinant today)
- h-choriogonadotropin - from the urine of pregnant women (today recombinant)
- hirudin - Medical leeches (*H. officinalis*) (today synthetic / recombinant)

MUNI
MED

# The production of biological drugs - recombinant technology

**3 generation of biologicals**

1 ) "copies" of human proteins

2 ) modified proteins (AAs substitution, glycosylation, PEGylation)

- better pharmacokinetics, pharmacodynamics - e.g. glargine, PEG-IFN

3 ) de novo designed proteins / MAB

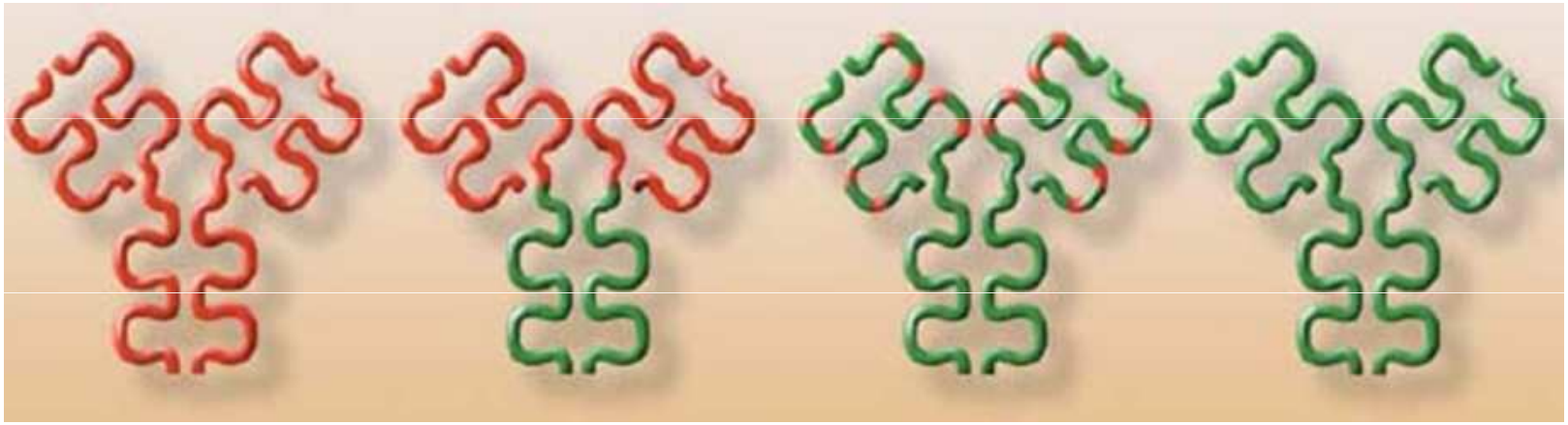
MUNI
MED

# Nomenclature

- Derived from biochemical name (Pegasys -PEG IFN)
- The name given by the manufacturer - unrelated to the effect of the origin
- Hormone with different tradename (Serostim, Saizen, Zorbtive)
- MAB- system root words and suffixes

# Nomenclature of MAB

- Generally: suffix -mab
- Letter before suffix:
  - o – of mice origin
  - a - rat origin
  - e - hamster origin
  - i - of primates
  - u – of human origin (human cell line production)
  - zu – humanized
  - xi – chimeric
  - mumab – fully human



**Mouse MAb**
100% of the mice orig.
Hypersensitivity
High levels of Ab
(not used clinically)

**Chimeric MAb**
34% of mice orig.
Hypersensitivity
Low levels of circulating Ab
(rituximab infliximab)

**Humanized**
5-10% mice orig. MAb
Hypersensitivity
Low levels of Neutralizing Antibodies
(Trastuzumab Certolizumab)

**Human MAb**
100% human
Hypersensitivity
Low levels of Neutralizing Antibodies
(panitumumab adalimumab)

MUNI MED

# Nomenclature of MAB

Sometimes encoding indication

- lim - immune
- bac - bacterial
- cir- cardiovascular
- tu - malignity

E.g.
rituximab - chimeric MAB to treat Non-Hodgkin. lymphomas

alemtuzumab - humanized antibody to the CD52 glycoprotein CLL

# The production of biological drugs - recombinant technology

- DNA extraction
- product / synthesis according to library
- transformation / DNA transfection into producer cells
- production
- purification
- stabilization
- testing (biological activity - CT I-III)
- registration ( RCT + IV )

# The production of biological drugs - recombinant technology

- DNA extraction
- product / synthesis according to library
- transformation / DNA transfection into producer cells
- production
- purification
- stabilization
- testing (biological activity - CT I-III)
- registration ( RCT + IV )

# Contaminants from manufacture process

- Microorganisms - antigenic structures, pyrogenicity , sepsis
- Viruses
- DNA - ? Consequences?
- Custom product in improper 3D structure
- Contaminating proteins
  - antigenicity
  - stability (protease )
  - safety (growth factors, hormones, toxins)

Purification - affinity gel / permeation chromatography

MUNI
MED

# Contaminants from manufacture process

Purification - affinity gel / permeation chromatography

Purity ± 98-99 %

→ **Verification of the biological activity of each batch !**

- Biochemical methods , cell lines or animal ( e.g. Epoetin )

= Time-consuming , cost , accuracy

MUNI
MED

# The production of biological drugs - recombinant technology

- Microorganisms
  - bacteria - optimized E. coli strains (mutations of periplasmatic and membrane protease)
  - yeast - S. cerevisiae
- Tissue cultures of higher organisms
- Cell-free expression systems
- Genetically modified animals, plants

MUNI MED

# The production of biological drugs - recombinant technology

***E. Coli***

- The synthesis of proteins **without posttranslational modifications**

- Cheap medium, mutated forms of E. coli with advantageous properties - increase the stability of the gene product ...

- Modification of wall, transformation of plasmid (DNA product introduction) - thermal shock, electroporation

- Selection - resistance to antibiotics / cell culture media

- Renaturation

- E.g. IFN , GSM, insulin, growth hormone ...

# The production of biological drugs - recombinant technology

***S. Cerevisieae***

- synthesis of proteins **with posttranslational modifications,** possibility of hybridization
- easy, economical cultivation, generation time of 2h, mutants with advantageous properties - increased stability of the gene product

- modification of cell wall, transformation of plasmid (various vectors)
- selection - auxotrophic strains (disabled biosynthetic pathway for AA, NA); plasmid introduce this gene – only transformed yeast are viable in selection media (ATB)

- renaturation
- E.g. insulin, growth hormone ...

# The production of biological drugs - recombinant technology

**Tissue cultures of higher organisms**

- About 60% of recombinant proteins

**positives:** same way of modifications as in humans

a wide variety of products

eliminates ethical / technical problems (isolation, animal cells, the lack of material)

**negatives**: higher risk of contamination

(rich medium, slower growth, expensive, difficult cultivation)

# The production of biological drugs - recombinant technology

**Tissue cultures of higher organisms**

Primary cultures (subculturing or passaging not possible)/ cell lines (tumor)
mostly adherent cell lines - release trypsin

Medium: ions , glucose, vitamins, nucleotides , lipids, calf serum (source of growth factors, hormones + PDGF, EGF, FGF, ... )
pH control, morphology

Vectors (details are kept secret): plasmids , viral plasmids (retroviruses), polycations

Part of the transfected DNA are regions of DNA increasing production

Selection (principles similar to those of S. cerevisiae . )

MUNI MED

# The production of biological drugs - recombinant technology

**Tissue cultures of higher organisms**

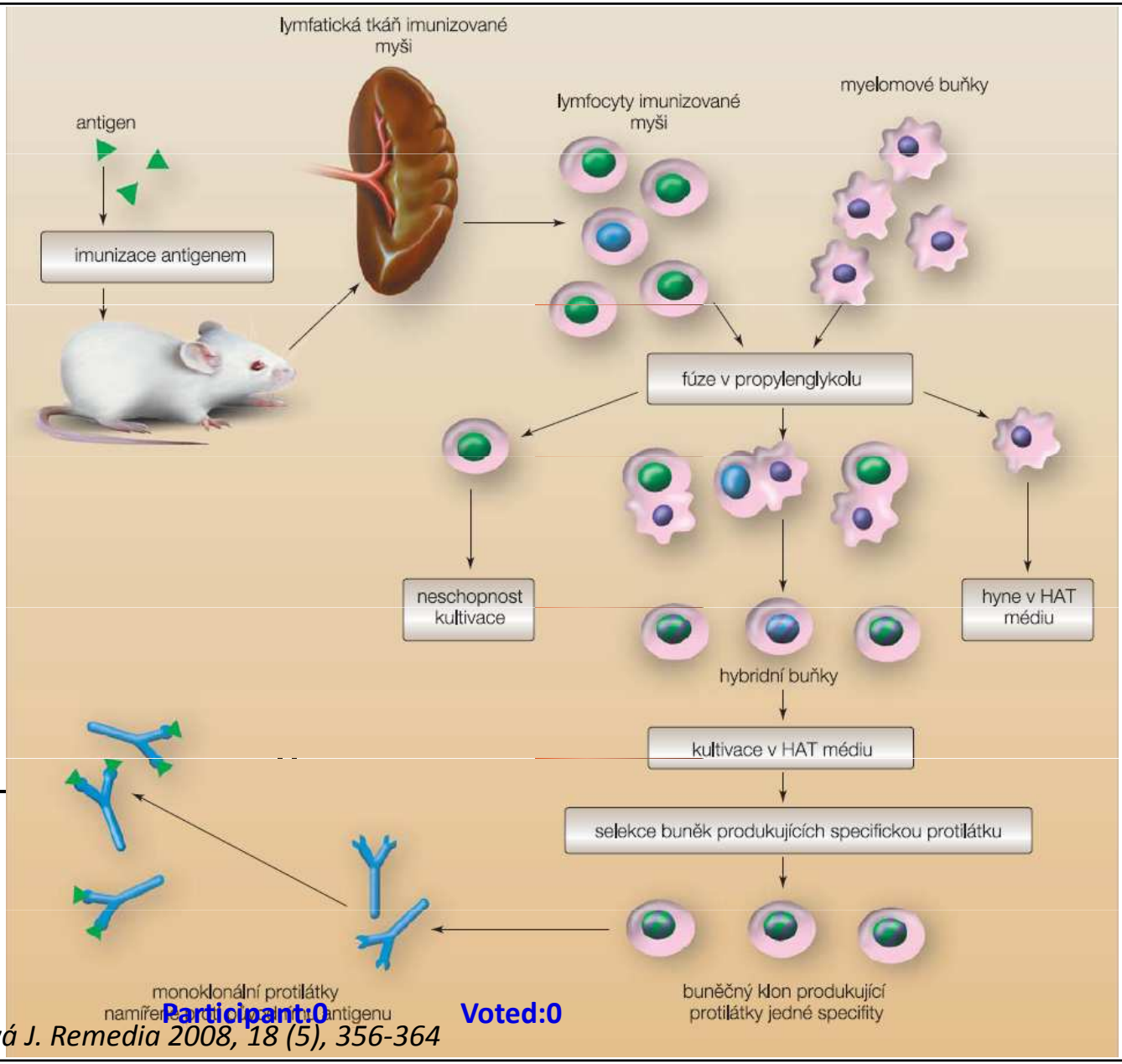
CHO – chinese hamster ovary

NS0, Sp2/0-Ag14 – mouse plasmocytome cells (leukocytes) – auxotrophic for L-glutamine

BHK 21 (baby hamster kidney – syrian hamster)

# Tissue cultures of higher organisms

production of „hybridomes“




*Sobotková M, Bartůňková J. Remedia 2008, 18 (5), 356-364*

# The production of biological drugs - recombinant technology

Transgenic plants

„**Edible vaccines** " - production of the immunogenic protein (like the polio vaccine )

culturing plant tissue culture in agar
*Agrobacterium* transfection (+ recombinant plasmid)
selection, planting

tobacco (Nicotiana tabacum ), Arabidopsis thaliana

**PRX - 112 - 06/2014** - 1st patient treated with recombinant protein from the plant ( Protalix Biotherapeutics ) Gaucher disease - deficit of glucocerebrosidase

„…active recombinant proteins systemically through oral administration of plant cells expressing biotherapeutic proteins...“

MUNI
MED

# The risk of functional damage of biologicals

- denaturation
- precipitation
- deamination
- mismatch of SH groups ( = incorrect 3D )
- oligomerization, aggregation, covalent binding
- hydrolysis
- isomerization
- racemisation
- formation imides
- oxidation

Multiple stabilizers
cryopreservation

metal chelation

checking the pH, osmolarity , strengthening the hydrophobic bonds

MUNI
MED

# Costs of the treatment

- Biological therapy is more expensive than "traditional" drugs

- Reasons - significantly higher development costs
  - Demanding and complex testing
  - The nature of products and higher costs after launch
  - Higher costs for production, storage, transportation, shorter expiration

  consequences: **lower numbers of treated patients** (up to 2 orders !!! )

**Despite that:**
- effective and in many cases can **save** money in terms of direct and indirect costs

*direct costs*: shorter hospitalization, reducing the number of surgical procedures, reduce the cost of follow-up treatment , ...

*indirect costs* : accelerating the patient's self-sufficiency, reducing the costs of absenteeism, cost reductions in social support and care allowances , reducing the cost of informal care and nursing

MUNI MED

# BIOSIMILARS

# BIOSIMILARS

" Copy" of biotechnology drugs

- produced after the expiry of patent protection on the original biotechnology drugs

- In the US, for the same group uses the term „Follow –on Biologics“ , abbreviated „fobs“

The standard procedure for the registration of generic medicines with defined structure (ie . bioequivalence study) is inapplicable

MUNI
MED

# BIOSIMILARS

- Biosimilars drugs are similar, but **not identical** with the original biological drug.
- Biosimilars are **not automatically therapeutically interchangeable** with the original biological drug .
- **small change** process in biosimilars may cause an **entirely different drug**.
- Biosimilars pass before entering the market or **shorter simplified clinical trials** , but disproportionately more complex than with generics

MUNI MED

# Biological drugs in a broader context

1. Gene therapy
2. Anti-sense therapy
3. Immunization with vaccines

MUNI
MED

# Biological drugs in a broader context

## 1. Gene therapy

- incorporation of a gene sequence into a target tissue by an appropriate vector
- treating or preventing gene-related illnesses by changing the expression human genes

**AE, risks:**

- Adverse **immune response**
- Infections vector - natural activation of **virus**
- Genetic influence on **gametes**
- Risk of **malignity**- activation of protooncogenes , suppression of regulatory genes

# Biological drugs in a broader context

## 2. Anti-sense therapy

- Incorporation of complementary oligonucleotides to the **initiation codon / promoter** to DNA
- block the effects of action of proteins that are not transcribed

- **Olimersen** - lowering expression of Bcl -2 (overexpressed in many cancer) - withdrawn from registration

# The antisense and gene therapy in practice

- **Fomivirsen** - antisense sequences to the mRNA of human CMV - Ophthalmic applications for pac . **HIV** + to reduce **CMV** infection

- **Pegaptanib** - oligonucleotide binding to the VEGF protein - for the treatment of **wet AMD**

- **Glybera** - 3 x 1012 genome copies of human lipoprotein lipase in a viral vector (adeno-associated virus serotype 1 (AAV1 ) to treat **hyperlipoproteinemia I**

# Biological/targeted treatment of selected diseases

1. Oncology
2. Rheumatic diseases
3. Psoriasis
4. Inflammatory bowel disease
5. Asthma
6. Multiple sclerosis
7. Ophtalmology
8. Hyperlipidemia

# Pharmacokinetics of biodrugs

- different than in „classic“ drugs (ADME)

- **administration parenteral**, absorption via lymphatic system, low bioavailability
- s.c. administration (not mAb)
- i.v. only in specialised centers, higher risk of immune reaction
- **Tmax** - several days!
- big molecules – slow and limited distribution, low Vd, binding to carrier proteins, no albumin!

# Pharmacokinetics of biodrugs

- different than in „classic“ drugs (ADME)

- elimination – not liver, mostly katabolism, intensive elimination via neutralising Ab (saturable)

- kidneys – small peptides, active tubular transport

Binding to target influences PK → The higher the dose, the lower Vd
The higher the dose, the lower Cl

# Adverse effects in general

**mAb**:

1. Reaction to foreign protein - allergic reaction, anaphylaxis (prevention – premedication, slow administration of the 1st dose)

2. Tumor lysis syndrome – typical for hematological malignities – ion dysbalance (hyperuricaemia, hyperkalaemia, hyperphosphataemia and hypocalcaemia)

3. Effect on healthy cells - rash, diaorrhea, tiredness, neurological symptoms, heart and lungs may be affected

# Adverse effects in general

**TKI**: decreased haematopoiesis, oedemas, fever, nauzea, vomiting, hematomas, rash, hair loss, bain in joints and muscles, changes in perception of taste and vision, dyslipidaemia…

In case of toxicity it is usually possible to decerase dosing without loss of clinical effect.

# 1. Biological (targeted) treatment in oncology

„target“ may be localised in

**tumor cells** - membrane receptor – extracellular part or/ intracellular signalling pathway

**immune system (specific T-cells)**

- cancer immunotherapy
- Immune check-point inhibitors (anti-CTLA-4 or anti-PD(L)1)

# 1. Biological (targeted) treatment in oncology

## Target on tumor cells

The most common targets

- **EGFR** *(epidermal growth factor receptor)* – **trastuzumab, pertuzumab, erlotinib,** lapatinib
- **VEGF***(vascular endothelial growth factor receptor)*- **bevacizumab, sunitinib**
- **PDGF** (*platelet derived growth factor receptor)*
- **FGF** (*fibroblast growth factor receptor)*
- **SCGF** = **c-KIT** *(stem cell growth factor)* - **imatinib**

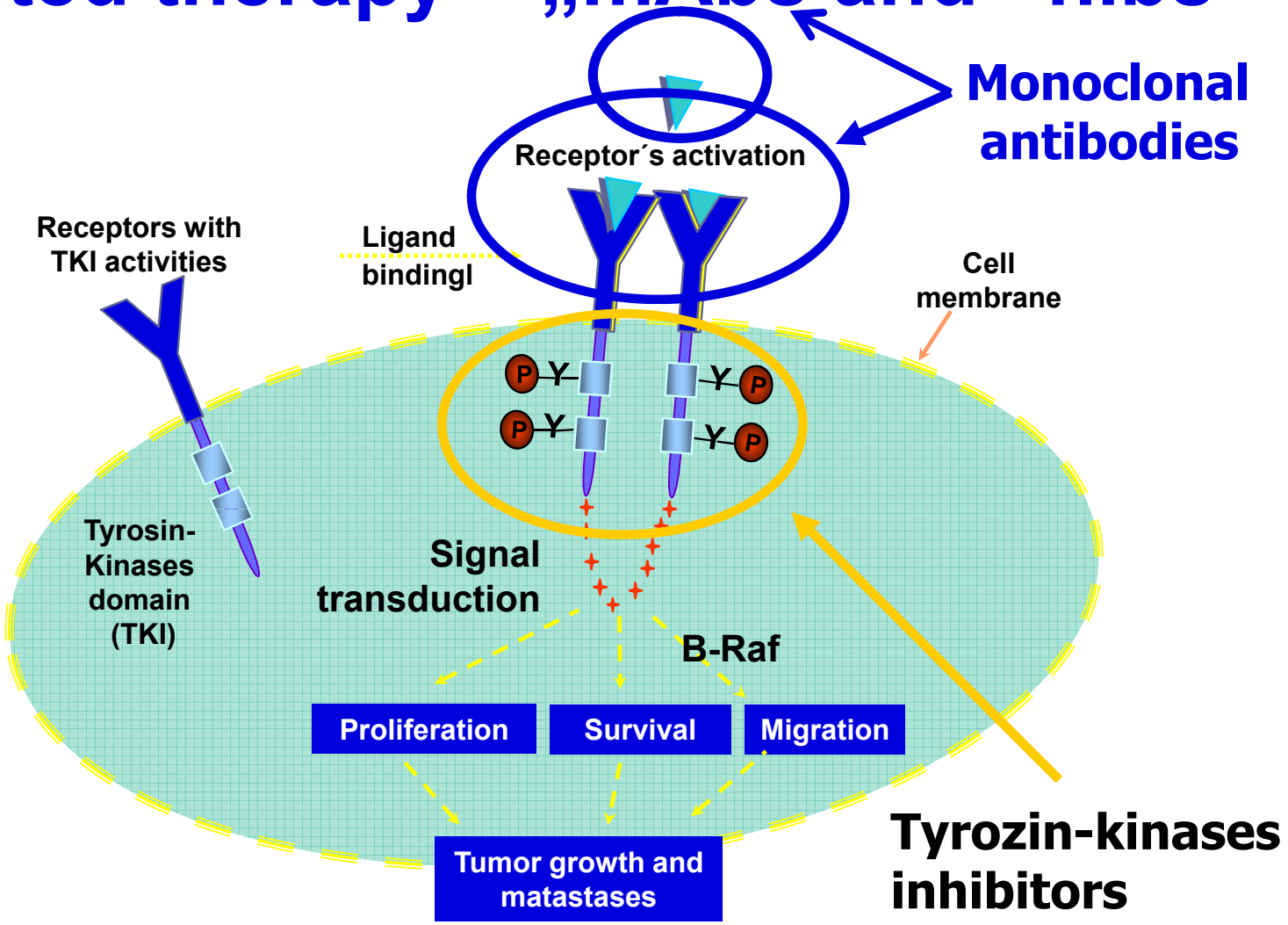
**MoA**:

- antagonization of extracelullar part of **receptor** or endogenous **ligand** - monoclonal antibodies (-mabs)

- inhibition of **intracellular pathway** – proteinkinase inhibitors (-nibs)

MUNI MED

# Targeted therapy – „mAbs and –nibs“



# Trastuzumab (HERCEPTIN ®)

## Target on tumor cells

HER-2 - 1985 – identification of the human Her-2/neu gene as a negative prognostic marker

**I:**

treatment of HER-2 positive breast cancer or adjuvant therapy of breast Ca

**AE:**

allergic reaction, fever, chills, hypotension

cardiotoxicity

diarrhea, nausea, vomiting, rash

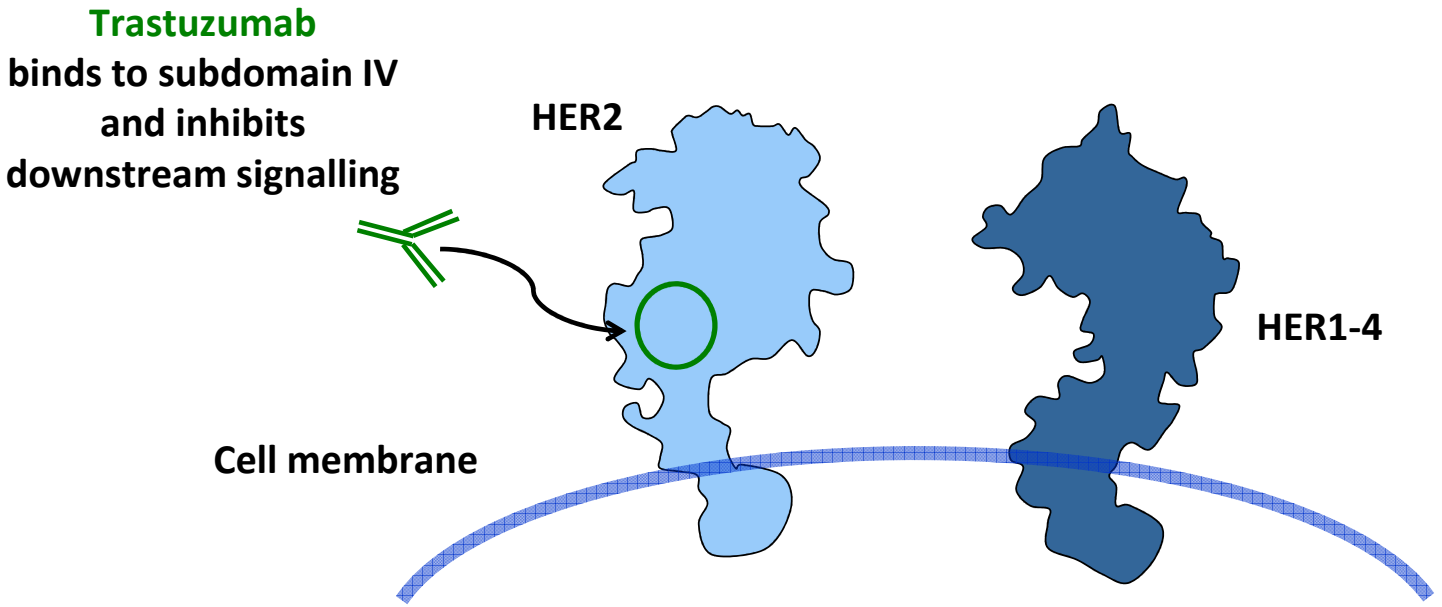
muscle and joint pain

pulmonary infiltrates, penumonitis

# Trastuzumab (HERCEPTIN ®)

## Mechanisms of Action

**Target on tumor cells**




Franklin MC, et al. *Cancer Cell*. 2004;5(4):317-328.

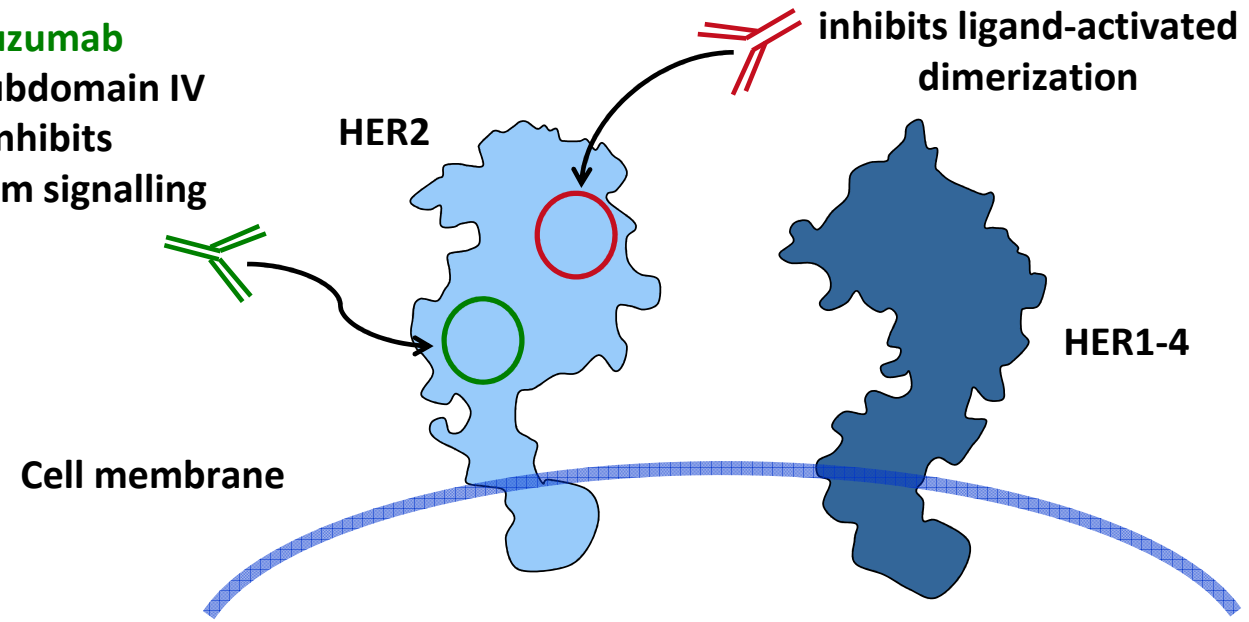
# Pertuzumab (PERJETA)

## Mechanisms of Action

**Target on tumor cells**

**Pertuzumab binds to a specific domain II and inhibits ligand-activated dimerization**

**Trastuzumab binds to subdomain IV and inhibits downstream signalling**




**The combined regimen of pertuzumab and trastuzumab offers the potential for a more comprehensive HER blockade**

Franklin MC, et al. *Cancer Cell*. 2004;5(4):317-328.

# Erlotinib

Target on tumor cells

**MoA:**

HER1 (EGFR – 1) TKI

**I:**

non-small-cell lung carcinoma (NSCLC)

pancreatic cancer

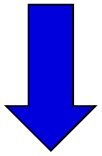
# Bevacizumab (AVASTIN ®)

## Target on tumor cells

The growth of malignant tumor needs the **continuous supply of oxygen and nutrients.**
Simple diffusion and not enough nutrition to the cells under the influence of hypoxia.
Tumor produced a series mediators, particularly **VEGF** (vascular endothelial factor).

**MoA:**

mAb against VEGF preventing it from binding to receptors



inhibition of angiogenesis and regression of tumor vasculature

MUNI MED

Target on tumor cells

# Bevacizumab (AVASTIN ®)

**I:**
Metastatic colorectal Ca
Metastatic breast Ca, renal Ca, non-small-cell lung Ca

**AE:**
acceleration of hypertenzion
proteinuria
trombembolic complications
poor wound healing

# Sunitinib

Target on tumor cells

**MoA:**

Multikinase inhibitor (anti VEGF, PDGF, c-KIT)

**I:**

GIST – gastrointestinal stromal tumor

mRCC – renal cell carcinoma

pNET – pancreatic neuroendocrine tumors

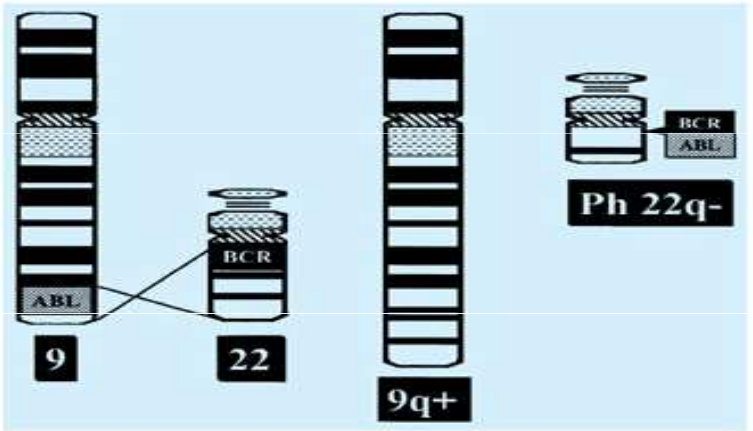
# Imatinib mesylate (GLIVEC ®)

**Target on tumor cells**

- Bcr-abl inhibitor – CML
- c-KIT inhibitor – 1st line treatment of GIST (mutation c-KIT in 85% pts.) – 70% of the pts. Are responders!!!

AE:

- neutropenia, trombocytopenia
- diarrhoea, vomiting
- joint pain






(BCR-ABL Translocation)

# Rituximab

**Target on B cells**

1997 - fhe first registered mAB approved by FDA for the treatment of lymphomas

**MoA:** binding to the transmembrane antigen CD20 (on pre-B and mature B lymphocytes), which is expressed on> 95% of all non-Hodgkin lymphomas of B cell origin

**I:** NHL, CLL, autoimmune disesases

AE: rash, itchiness, hypotension

severe – infection, toxic epidermal necrolysis

# „Checkpoint inhibitors“

**Target on T cells**

**Checkpoints**

– provide protection from immune destruction even during an immune reaction

- may have stimulatory on inhibitory function

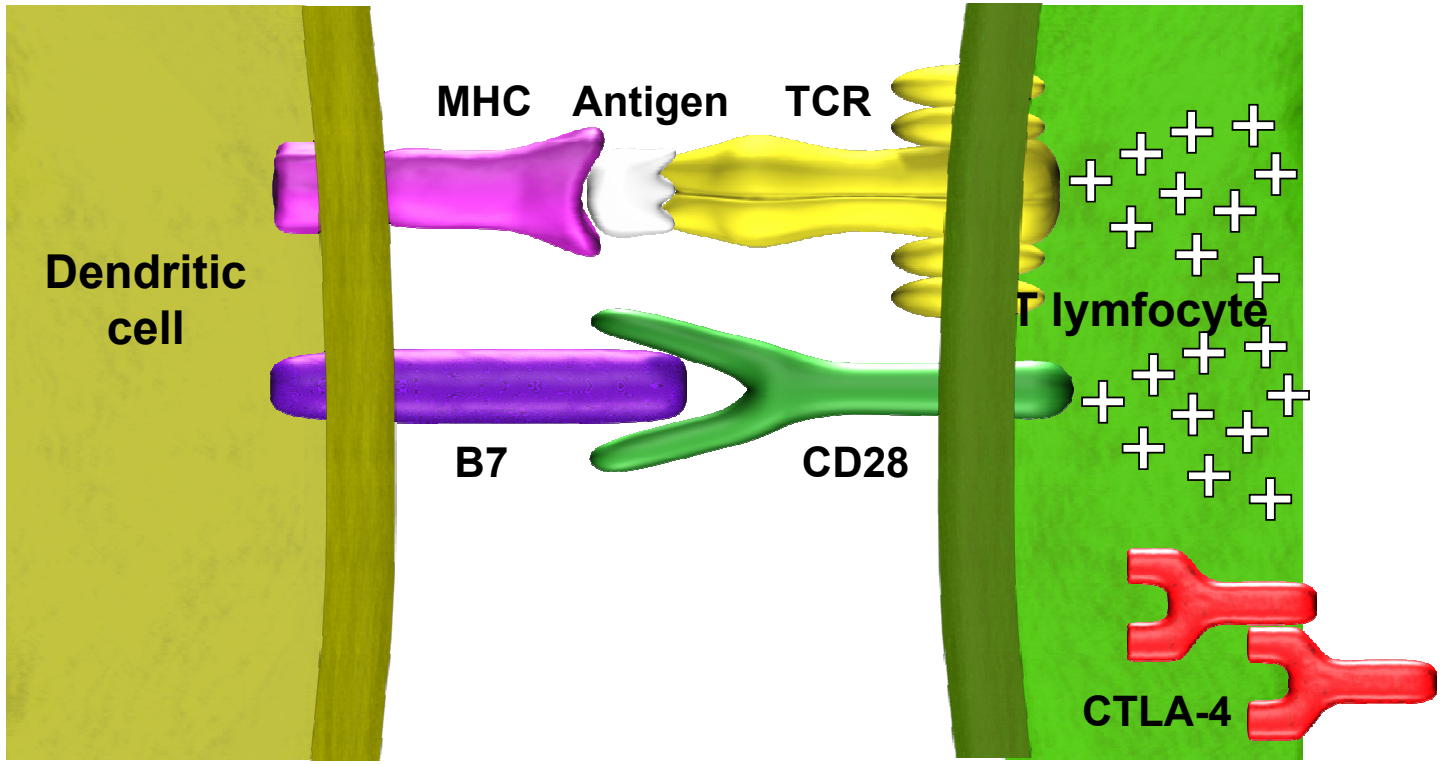
**anti-CTLA-4 (cytotoxic T-lymphocyte antigen 4) – co-inhibitory**
**– ipilimumab -** melanoma

**anti-PD-1** (programmed death-1 receptor) - **nivolumab, pembrolizumab**
- melanoma, RCCa, NSCLC

**anti-PD-L1** – atezolizumab

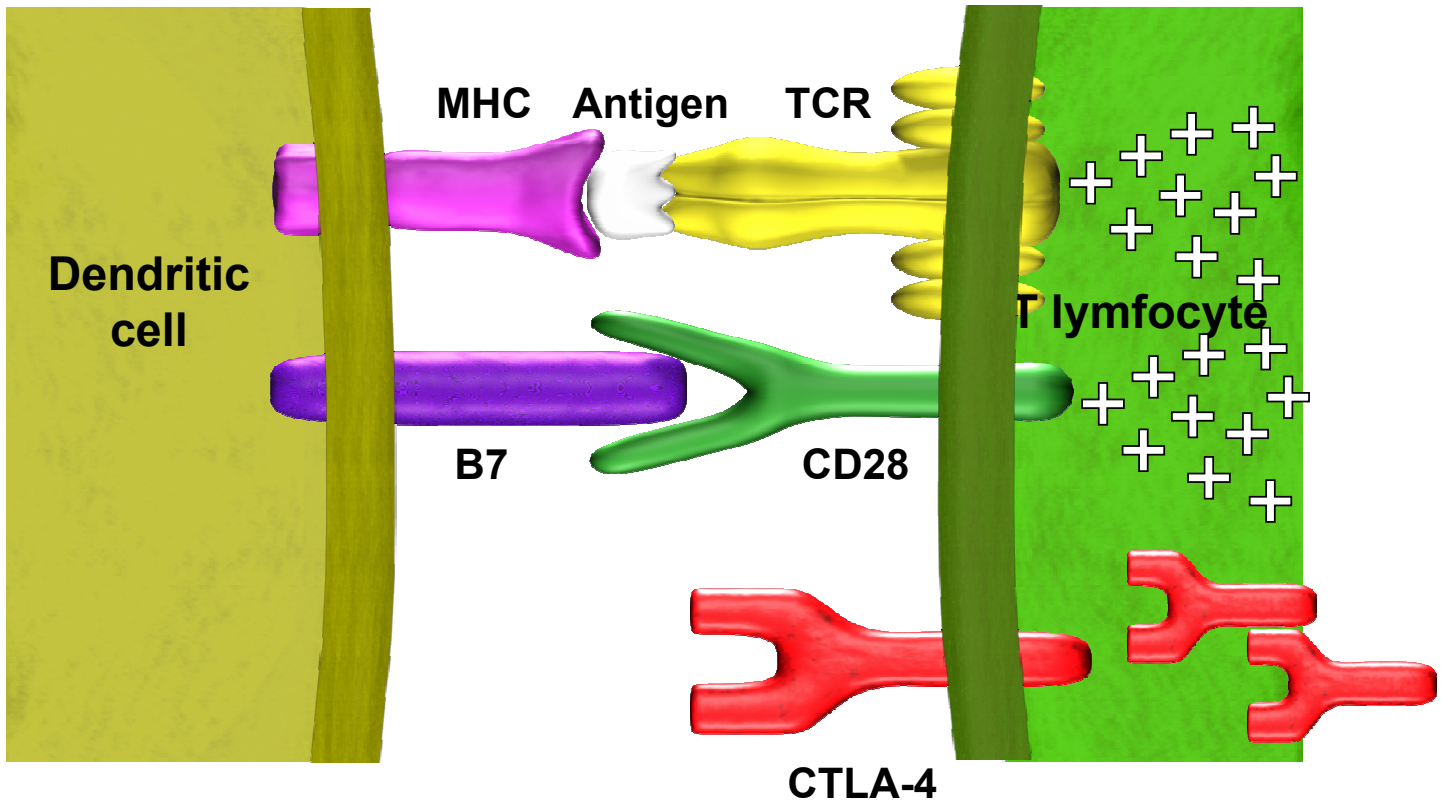
MUNI MED

# Activation of T lymfocytes through TCR and co-stimulating molecule CD28



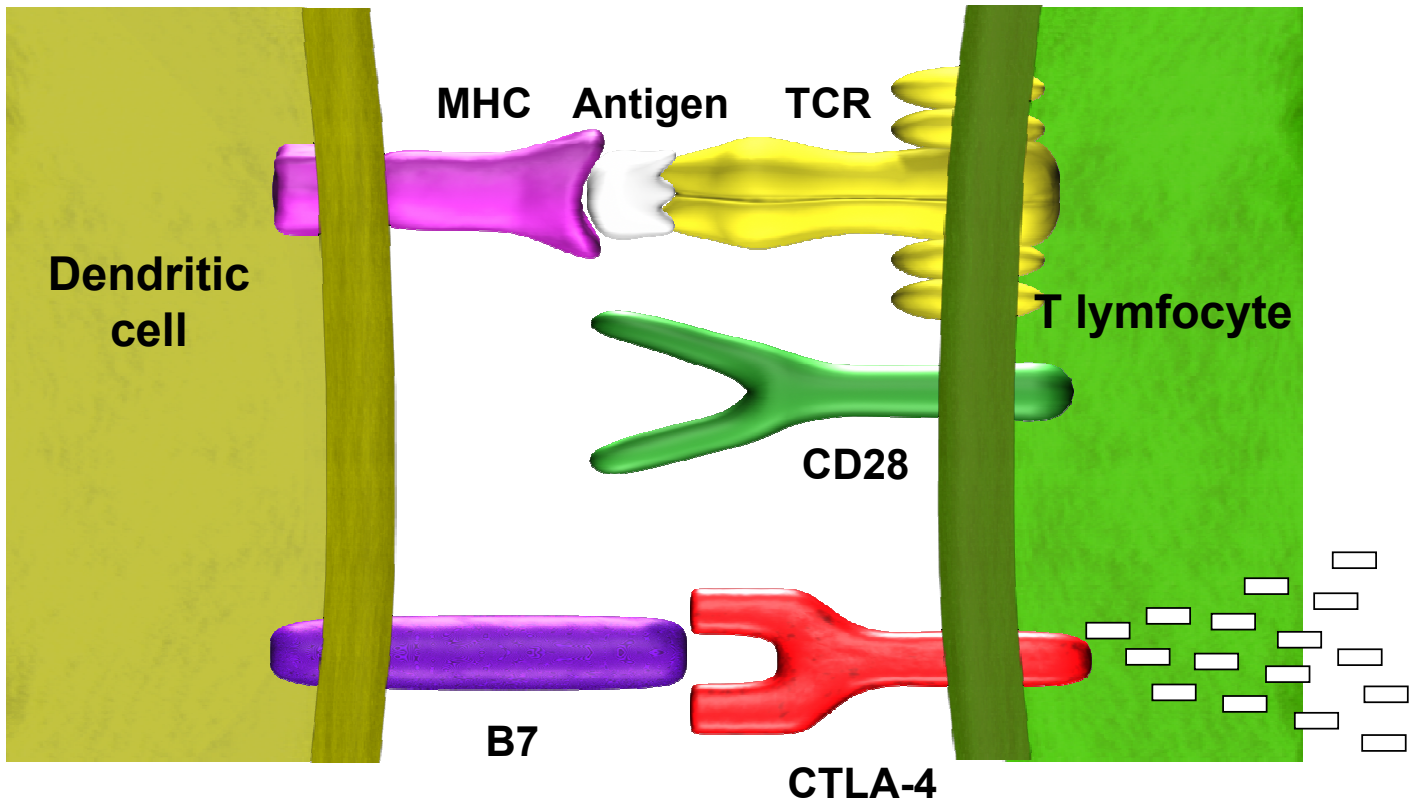

MUNI MED

# Up-regulation of CTLA-4 receptors after T- cell activation



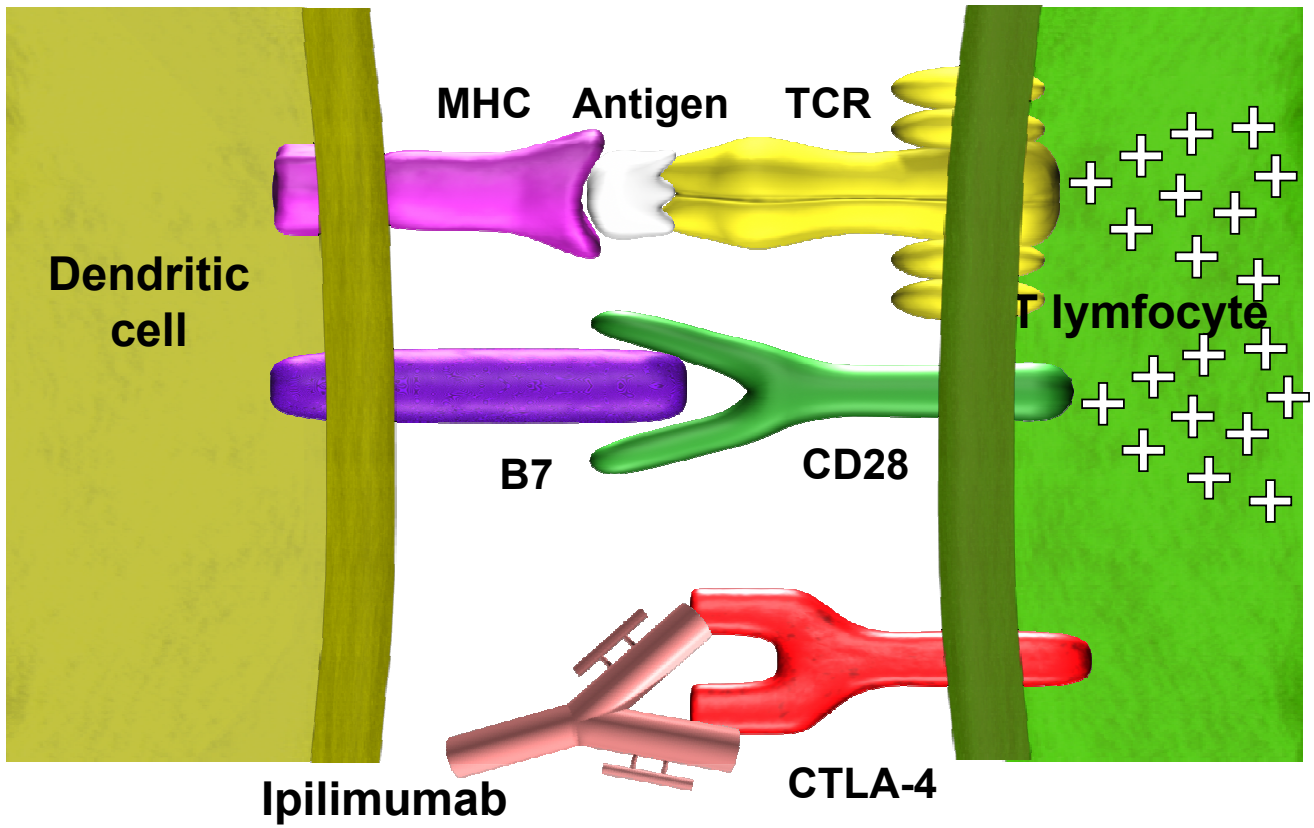

MUNI MED

# CTLA-4 receptor inhibition




MUNI MED

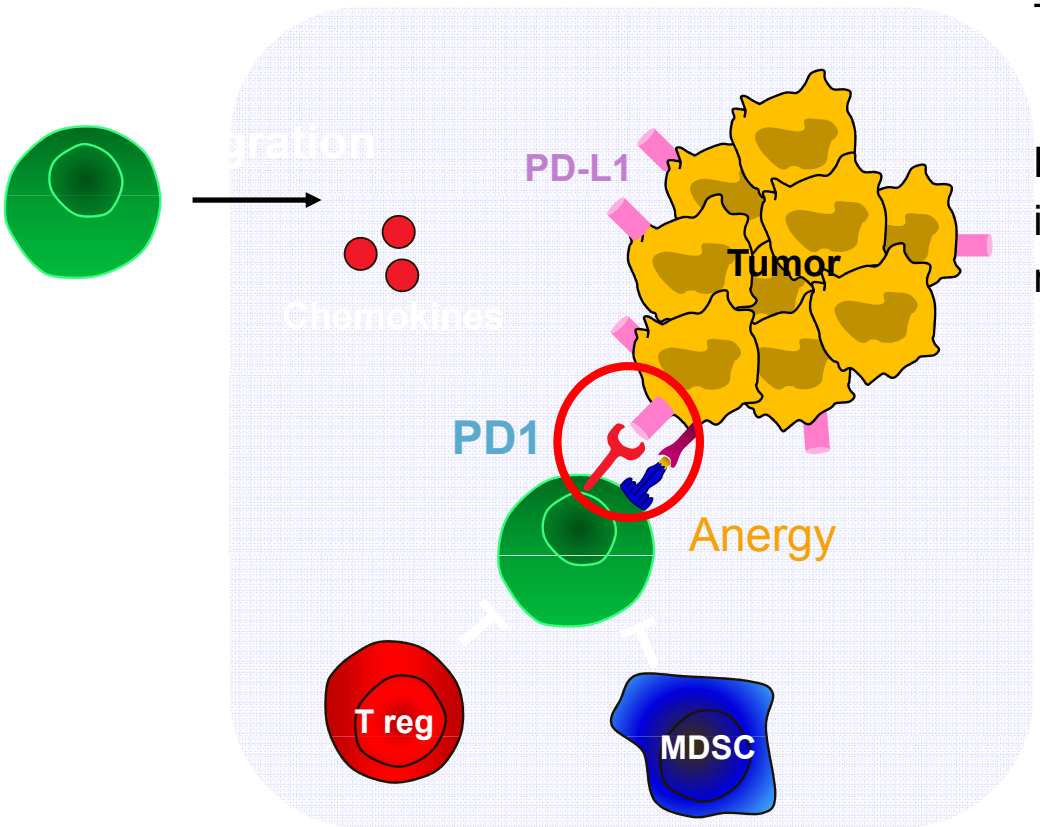
# Antagonisation of CTLA-4 receptors Ipilimumab




Leach DR, Science 1996;271:1734-1736.

MUNI MED

# Checkpoint inhibitors – PD-(L)-1




T cell recruitment

Blocking PD1:PD-L1 binding might activate immunity within the tumor microenvironment

Gajewski TF, et al. Curr Opin Immunol. 2011;23:286-292. Spranger S, Gajewski T. J Immunother cancer. 2013;1:16.

## 2. Biological treatment of rheumatic diseases

**Anti-TNF drugs** – Ab (infliximab, adalimumab)

- receptor etanercept

**Anti IL drugs** - anakinra, tocilizumab

**Immunomodulants** – adalimumab, rituximab

## 2. Biological treatment of rheumatic diseases

### Anti-TNF drugs - Ab

**Infliximab** – chimeric mAbs, IgG, 75 % of human, 25% of the murine antibody
high affinity binding to human TNFα

**Adalimumab** - human MAB binds specifically to TNF and neutralizes the biological function of TNF by blocking its interaction with the p55 and p75 cell surface TNF receptors

Certolizumab – humanized Fab fragment of TNFα antibody, conjugated with PEG

Golimumab - the same mechanism action as infliximab, not registered in EU

# 2. Biological treatment of rheumatic diseases

## Anti-TNF drugs - receptor

**Etanercept** – soluble dimeric fusion protein – extracelular domain of receptor for TNFα and Fc chain of human $IgG_1$

Mechanism of action: competitive inhibition of TNFα, decreased effect of TNFβ

does not bind complement, but leads to the disintegration of granulomas

## 2. Biological treatment of rheumatic diseases

### Anti-TNF drugs – adverse effects

**Opportunistic infections –** increased risk in combination of immunosuppressive drugs (with 2 - combined to 14x ! ), malnutrition, age > 50 years

- mycobacteria , listeria , fungal , viral infections

**Paradoxical autoimmune reactions**

**Anti-idiotypic antibodies** (in addition prevents binding of the antibody to TNF)

**TB** –activation of latent forms

**Late carcinogenicity** - lymphoproliferative disease (2-3 times higher versus the healthy population) , inconsistent data

**Others** - specific AE for specific substances

## 2. Biological treatment of rheumatic diseases

### Anti IL treatment

**Anakinra** – IL1 receptor antagonist

weaker effect than anti-TNF drugs

**Tocilizumab** - humanized mAb against IL6

AE: (infection ) + increase in lipids ( CHOL , LDL, TAG)

## 2. Biological treatment of rheumatic diseases

### Immunomodulants

**Abatacept** - recombinant fusion protein from composed of Fc region of IgG and extracellular domain of CTLA4 ( receptor expressed in T-cells); competitively binds to CD80, preventing T cell activation proliferation

**Rituximab** - binds to the transmembrane antigen CD20 (on pre-B and mature B lymphocytes) expressed on> 95% of all non-Hodgkin lymphomas of B cell origin

# 3. The biological treatment of psoriasis

## Anti-TNF drugs

**Etanercept** - see above

- Only one biological treatment of psoriasis for children 8-18 years

**Infliximab** - see above

## Anti IL drugs

**Ustekinumab** - fully human MAb IgG1 anti-IL -12/ 23 (important in the pathogenesis of psoriasis), inhibition of cytokine cascade

AE : nasopharyngitis, headache, arthralgia, local irritation at the injection site

MUNI MED

# 4. Biological treatment of inflammatory bowel disease

(Crohn's disease, ulcerative colitis)

## Anti-TNF drugs (see above)

- infliximab
- adalimumab
- certolizumab

## Selective adhesion molecule inhibitors

**Natalizumab** – humanized IgG4 mAb against integrin α (prevents migration of leukocytes across the capillary wall)

**Vedolizumab** - humanized IgG1 mAb against α4β1 integrin (on activated leukocytes, provides adhesion to the endothelium and the penetration into the circulation from the gastrointestinal tract )

MUNI MED

# 5. Biological treatment of bronchial asthma

Adjunctive/supplementary treatment in patients with more serious desease which do not respond to other treatments

**Mepolizumab** - anti IL-5 Ab

**Omalizumab** – humanized Ab anti IgE

# 6. Biological treatment of multiple sclerosis

**IF β – 1a** – antiinflammatory, immunomodulatory effects, supresses Th1 T lymphocytes activity and HEB permeability

AE: flu-like syndrome, inhibition of hematopoiesis

**Natalizumab – see above**

**Anti-CD20 mAb (B cells) - rituximab (see above),** ocrelizumab, alemtuzumab

**Anti-IL-2 - daclizumab** humanized mAb

# 7. Biological treatment in ophtalmplogy

## Monoclonal antibody against VEGF - A

**Bevacizumab (see above) -** was developed for the treatment of colorectal cancer, off-label use in **wet age-related macular degeneration (AMD)**

**Ranibizumab**

Indications : AMD, CNV (chorioidal neovascularization)

# Thanks for your attention