VOLGOGRAD
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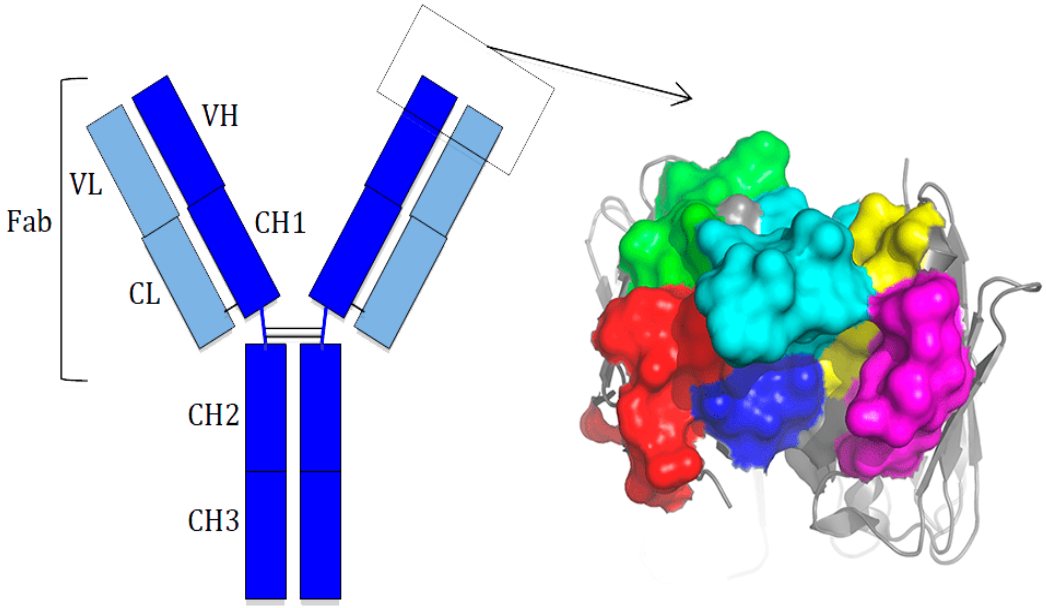
MONOCLONAL ANTIBODIES

Faculty of Pharmacy

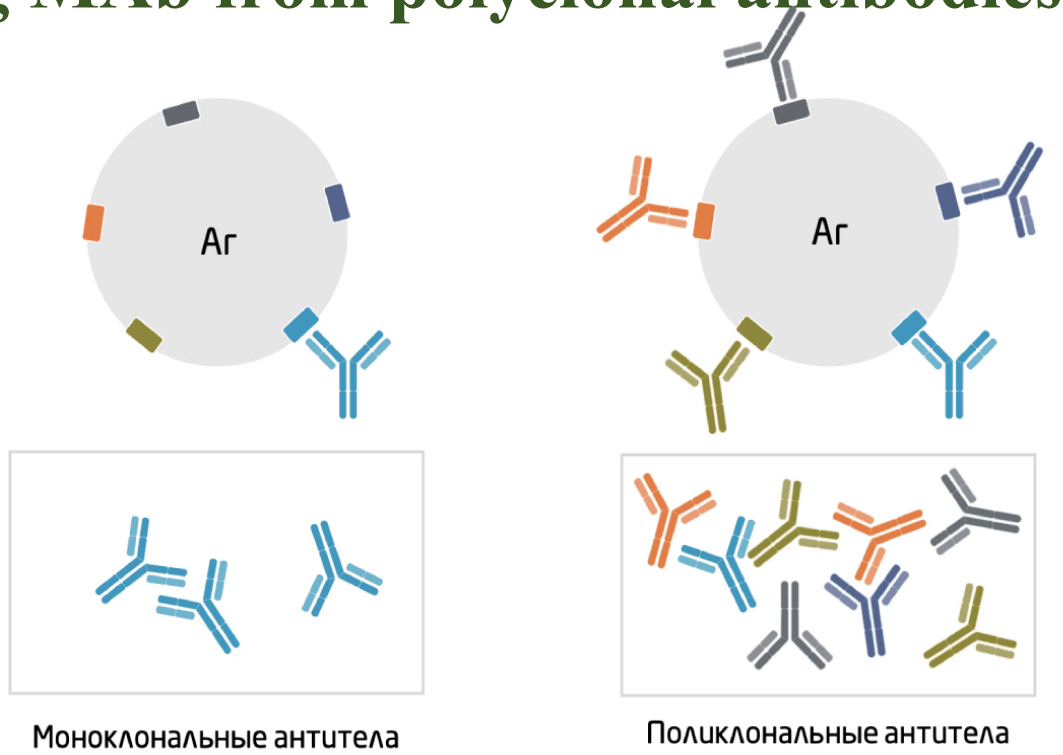
A monoclonal antibody (MAb) is an immunoglobulin with target specificity to a certain antigen produced by a stable cell line. The cells of a clone are identical and the antibodies produced by them have the same structure and properties.

Antibody structure ensures the ability of antibodies to recognize specific antigens (carried out by V-domains) and interact with cells of the own immune system (effector function of antibodies; C-domains are responsible for it).



Fab- fragment antigen binding - the part of the Ig molecule that binds the antigen. It consists of one constant (C) and one variable (V) domain of light and heavy chains. These domains bind the epitope of specific antigens

Distinguishing MAb from polyclonal antibodies



MAb contain the product of a single clone of plasma cells, directed to a strictly defined antigenic determinant, always having the same physical and chemical characteristics and affinity to the antigen, characterized by the **highest specificity**, **standardization and manufacturability of production.**

Polyclonal antibody drugs – a heterogeneous family of antibodies produced by different clones of plasma cells to different sites of the same antigen, contain a wide variety of antibodies that differ in their **specificity, affinity and physico-chemical properties**

History of the development and stages of monoclonal antibody production

In 1975, German immunologist **George Kohler**, who studied the genetic variability of antibodies, and British immunologist **Cesar Milstein,** who studied clones of tumor cells (plasmacytomas), created a hybrid of antibody-producing plasma and tumor cells.

They were awarded the **Nobel Prize** in 1984 for the development of this technology.

Monoclonal antibodies were first produced from a hybrid cell (hybridoma) derived from

- **an antibody-forming** B-lymphocytes stimulated with a specific antigen
- **an myeloid** (tumor cells originating from plasma cells-**plasmocytoma**), capable of unlimited multiplication under artificial conditions.

The hybrid cell had the **immortality** of a tumor cell and the **ability to synthesize** antibodies inherited from a normal cell.

Stages of the hybridomas production

1. **Immunization** of mice with the selected antigen. At the antibodies peak, the spleen was removed from the animals and the tissue was homogenized to obtain a suspension of B-cells, antibody producers against the injected antigen.

2. **Cultivation of tumor cells.** For hybridization with B-lymphocytes, only mutant plasmacytoma (myeloma) cells were selected, which had only the **main pathway** of nucleotide synthesis (from amino acids) and lacked the enzyme that provides nucleotide synthesis through the subsidiary pathway (from purines and pyrimidines)

3. **Fusion of tumor cells with "normal" lymphocytes.** Spleen cells were mixed with plasmacytoma cells in the presence of polyethylene glycol (PEG), a polyelectrolyte that promotes cell membrane fusion and the formation of hybrid cells. The hybridoma retained the ability to cell division, during which the chromosomes of both nuclei intermingled to form one common nucleus containing genes of both progenitor cells.

Stages of the hybridomas production

4. Hybrid selection.

Metabolic selection of hybrids was based on the fact that B-lymphocytes can use two metabolic pathways for purine and pyrimidine nucleotide synthesis: the **main** (from amino acids and carbohydrate precursors) and **reserve** (from **hypoxanthine (purines) or deoxythymidine (pyrimidines**).

If the main metabolic pathway of nucleotide synthesis is blocked, the enzymes **hypoxanthine-guanidine-phosphoribosyltransferase (G/GFRT**) and **thymidine kinase** are activated, allowing nucleotide synthesis via the reserve pathway. To separate a given hybridoma from other cells, a mixture of cells was placed in a selective medium (HAT medium) containing **hypoxanthine, aminopterin and thymidine**, in which the major pathway of nucleic acid synthesis was blocked (at the expense of toxic **aminopterin**), resulting in the death of mutant tumor cells that lacked the reserve pathway of nucleic base synthesis.

B-lymphocytes which are capable of growing in **HAT medium**, being lethal, died naturally after 1-2 weeks.

Only **hybridoma cells** combining the properties of “immortal” tumor cells and B-lymphocytes survived in the selective medium

Stages of the hybridomas production

5. **Cultivation of the produced hybridomas** in order to isolate a clone producing the necessary antibodies.

Cells surviving in HAT medium were placed in plastic 96-well plates (10 hybridomas in each well). After a few days of culturing, cells containing antibodies of a given specificity were cloned (reintroduced into wells at the rate of 1 cell per 1 well). The progenitor cell gave rise to the forMAbion of an "immortal" clone producing monoclonal antibodies.

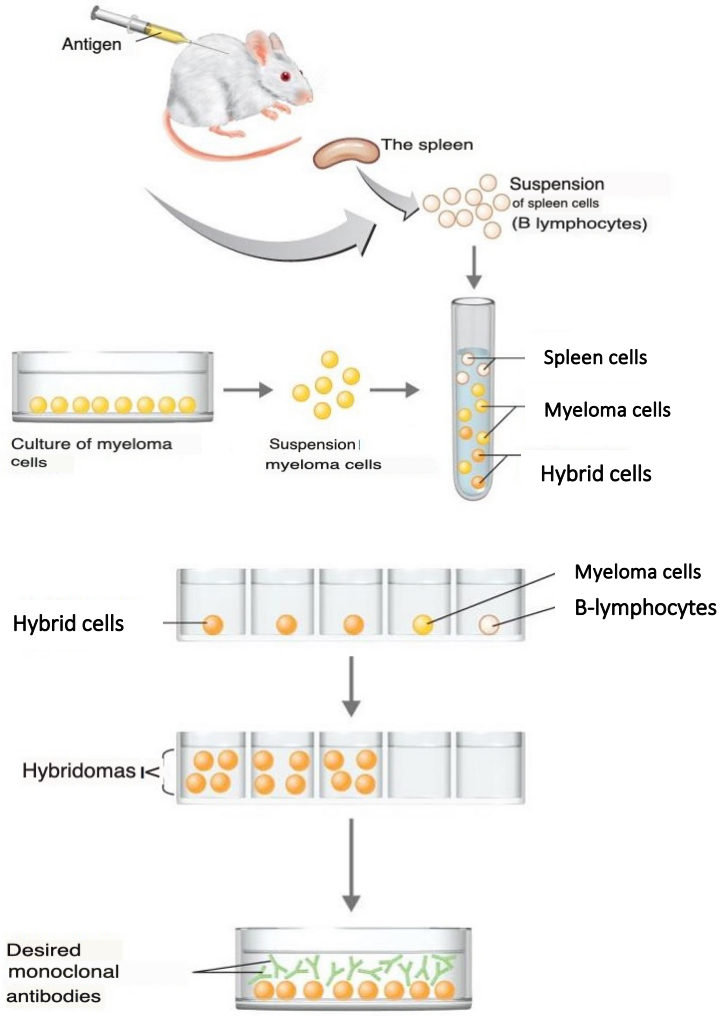
6. **Study of the produced hybridomas** - determination of specificity, affinity, etc.

7. Production of monoclonal antibodies *in vivo* (by grafting clones into mice) or in vitro (by culturing clones in culture medium).

8. Purification of the resulting antibodies.

Selected hybridomas can be cultured for long periods of time to obtain large amounts of homogeneous monoclonal antibodies Affinity and ion exchange chromatography are used for purification.

The main stages of producing a hybridoma.



1. **Mice are injected with a specific antigen, which causes them to produce antibodies against that antigen.**

2. The spleen of the mice is removed and homogenized to produce a cell suspension. This suspension contains B cells, which produce antibodies against the injected antigen.

3. The spleen cells are then mixed with myeloma cells, which are able to grow continuously in culture and also lack the reserve pathway for nucleotide synthesis.

4. Some of the antibody-producing spleen cells and myeloma cells fuse to form hybrid cells. These hybrid cells are now able to grow continuously in culture and also produce antibodies.

5. The cell mixture is placed in a selective medium that allows only hybrid cells to grow. Unfused myeloma cells and B lymphocytes die.

6. The hybrid cells proliferate, forming a clone of hybridomas. Hybridomas are tested for the production of the desired antibodies.

7. The selected hybridomas are then cultured to obtain large quantities of monoclonal antibodies.

Types of monoclonal antibodies

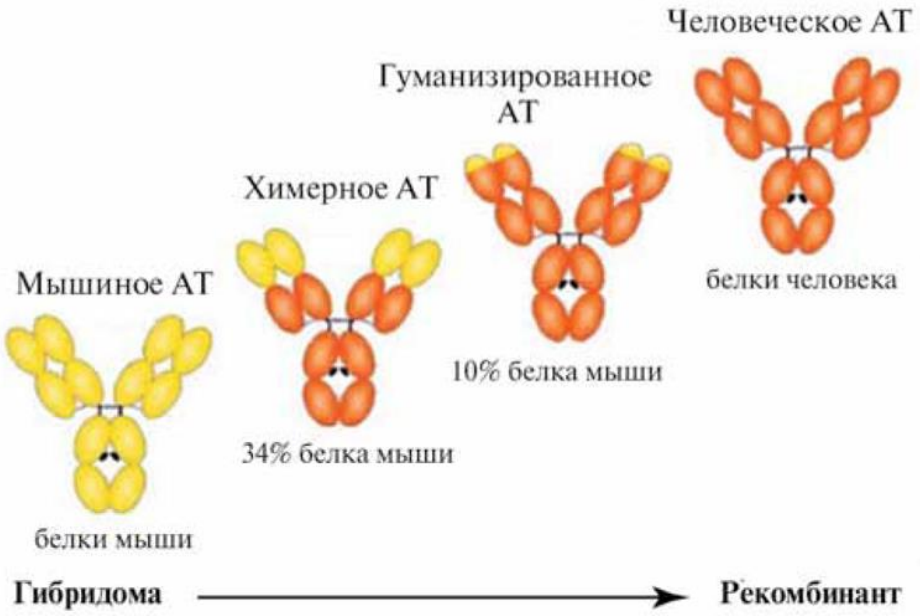
Mouse antibodies consist entirely of mouse protein, have high immunogenicity, are not effective enough in realizing the mechanism of target cell destruction, and are quickly eliminated from the body. In **1988**, British biochemist **Greg Winter** developed a method of "**humanizing**" monoclonal antibodies.

Chimeric antibodies are antibodies in which its constant domain, which has immunogenic and effector properties, is replaced with human immunoglobulina, while the variable domain, which specifically interacts with antigen, will remain murine.

Humanized antibodies are 90-95% human immunoglobulin. Only hypervariable regions responsible for binding of AT to antigen remain in them.

Recombinant human antibodies, in which the variable domains of heavy and light chains of human antibodies are combined with constant domains of human antibodies.

Human MAb have the lowest immunogenicity



Nomenclature monoclonal antibody preparations

Immunogenicity		Type	Consistency percentage, %		INN ending
			Mouse	Human	
Reducing	↓	**mouse**	**100**	**0**	**-omab** *murunomab*
		chimeric	**33**	**67**	**-ximab** *rituximab*
		humanized	**5-10**	**90-95**	**-zumab** *daclizumab*
		human	**0**	**100**	**-umab** *sarilumab*

Mechanism of action of MAb

-blocking a specific target that plays a role in disease pathogenesis, excretion or neutralization of the pathogen.

-blocking the pro-inflammatory activity of cytokines

-inhibition of the processes of activation and interaction of immunocompetent cells

-elimination of subpopulation of immune cells involved in inflammation.

First studied and described clinical targets of MAb

- tumor necrosis factor alpha (**TNFα**) (involved in systemic autoimmune diseases)

- pro-inflammatory cytokines (**interleukins**) involved in inflammatory, allergic and autoimmune reactions

- **CD20 protein** present on the surface of B-lymphocytes (in B-cell lympho-proliferative diseases)

- epidermal growth factor (**EGFR**), the increased expression of which can be observed in cancers

- human epidermal growth factor receptor 2 (**HER2**), which is overexpressed in breast cancer.

Therapeutic use of MAb

- therapy of chronic diseases with a long, progressive course

- **cancer immunotherapy**

- **autoimmune diseases** (rheumatoid arthritis, systemic lupus erythematosus, psoriasis, multiple sclerosis)

- **allergology** (for treatment of resistant, severe forms of bronchial asthma)

- **transplantology** (for prevention of transplant rejection)

- **therapy of viral, chronic inflammatory and orphan/rare diseases** (paroxysmal nocturnal hemoglobinuria, cryopyrin-associated periodic syndromes).

Applications of monoclonal antibodies

Identification of human lymphocyte subpopulations by immunophenotyping is based on the detection of differentiation markers (CD-antigens) on their surface, unique for each subpopulation and stage of development.

Using fluorochrome-labeled monoclonal antibodies that bind to specific CD antigens, lymphocytes belonging to different subpopulations are counted to assess the cellular immunity.

Diagnostic testing - monoclonal antibodies are used in the diagnosis of various diseases to test for the presence of foreign antigen (toxins, drugs, hormones, proteins of bacteria or viruses).

Serologic identification of ABO blood groups

Monoclonal antibodies can also be used for serologic identification of blood groups. Antibodies can be isolated from human sera stimulated with blood group A or B blood groups

Applications of monoclonal antibodies

Radioimmunodetection (RID) - using monoclonal antibodies, radioactive substances can be delivered to the tumor and its metastases, allowing detection of small tumor nodules by the localization of radioactivity in them.

Embryonic development analysis - determination of fetal sex, chromosomal abnormalities, single gene abnormalities

Quadroma -antibodies that have active centers to different antigens.

Immunopurification - a method of purifying individual interferons, proteins, and enzymes.

Side effects of MAb

- **Immunogenicity**-infusion reactions, anaphylaxis, reduction of the therapeutic effect of the drug substance

- possible development of a cascade of biological reactions accompanied by release of cytokines interleukin-6, TNFα, interferon (chills, flu-like syndrome, myalgia, etc.).

- increased risks of oncologic and viral diseases, since MAb cause suppression of the activity of certain parts of the immune system.

- long-term therapy with MAb (TNFα inhibitors) may result in reactivation of latent tuberculosis process, increased risk of infections, lymphoproliferative diseases, autoimmune reactions.

- decrease in the severity of clinical effect due to neutralizing action of antibodies or formation of immune complexes

Application of monoclonal antibodies in oncology

Two types of MAb are used in tumor therapy:

Simple (unconjugated) MAbs - acting directly on tumor-associated antigens, triggering the natural mechanisms of the body's immune response, destroying tumor cells through cytotoxic action or blocking proliferation and growth of tumor cells

Conjugated MAb drugs - a complex of antibodies specific to tumor-associated AGs, the therapeutic effect of which is due to binding to active substances (isotope, cytostatic or toxin) that are delivered to tumor cells.

Mechanisms of antitumor action of therapeutic monoclonal antibodies

1. **Immune mechanisms**

- induction of antibody-dependent cell-mediated cytotoxicity (ADCC)

- Induction of antibody-dependent phagocytosis (ADP)

- Induction of complement-dependent cytotoxicity (antibody-mediated complement activation) (CDC).

2. **Non-immune mechanisms**

- alteration of cell signaling (blockade of growth factor receptors necessary for cell division)

- induction of apoptosis

Immune mechanisms of antitumor action of therapeutic monoclonal antibodies

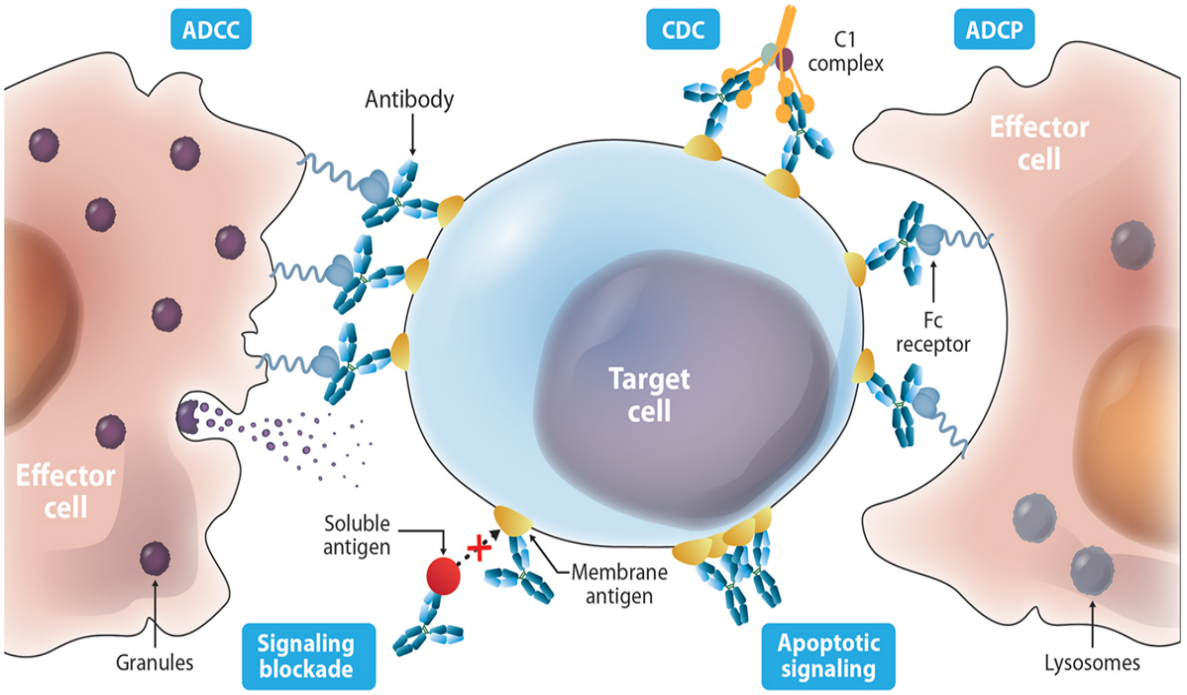
- **Antibody-dependent cellular cytotoxicity (ADCC)**
- **Monoclonal antibodies activate ADCC through recognition of their Fc-domain by receptors located on the surface of effector cells** (natural killer cells) that recognize tumor cells, resulting in the release of cytotoxic granules that cause lysis of tumor cells **(trastuzumab,* rituximab*, alemtuzumab*).**
- **Complement-dependent cytotoxicity (CDC)**
- In CDC, **a multistep proteolytic cascade is formed** that results in the death of foreign cells through the assembly of a membrane-associated complex (MAC). The antibody binds to the antigen on the surface of the target cell, which leads to activation of the complement system by the classical pathway. The formation of membrane-associated complex and the subsequent release of anaphylins and opsonins leads to lysis and phagocytosis of target cells (**rituximab*, ofatumumab*).**

Immune mechanisms of antitumor action monoclonal antibodies

Antibody-dependent phagocytosis (ADP)

Macrophages (and monocytes) phagocytize tumor cells in the presence of MAb.
ADP is enhanced after opsonization with antibodies because macrophages express specific Fc receptors.

The monoclonal antibody binds with its hypervariable domain to the corresponding antigen on the tumor cell surface, and with its constant domain - to the Fc receptor of cytotoxic lymphocyte, proteins perforins and proteases are synthesized and secreted, damaging the cell membrane, causing its lysis.



Non-immune mechanisms of antitumor action monoclonal antibodies

- **Alteration of cell signaling**

Some malignant cells have a large number of receptors on their surface for growth factors **(EGFR, VEGF-A, HER2),** which activate a cascade of reactions aimed at increasing cell proliferation. Blocking these receptors with a monoclonal antibody leads to tumor cell death.

(cetuximab*, necitumumab* (against EGFR), (trastuzumab*, pertuzumab (against HER2), (bevacizumab* (against VEGF-A))

- **Inducing apoptosis**

MAbs can induce apoptosis via the intrinsic (mitochondrial) pathway, leading to the release of cytochrome C from mitochondria and inhibiting anti-apoptotic proteins (rituximab), or increase the expression of a pro-apoptotic multidomain protein (**cetuximab***)

Classification of antitumor monoclonal antibodies by mechanism of action on target cells

I Monoclonal antibodies specific to antigens expressed on tumor cells (CD20 protein)

Rituximab*, Obinutuzumab*, Ofatumumumab*

II Monoclonal antibodies that affect growth factors.

1.Drugs that block the activity of epidermal growth factor (EGFR, EGFR2)

Cetuximab*, Panitumumab*

2.Monoclonal antibodies specific to the human epidermal growth factor receptor 2 (HER2)

Trastuzumab*, Pertuzumab*

3.Monoclonal antibodies blocking the activity of vascular endothelial growth factor (VEGF)

Bevacizumab*, Aflibercept*

Classification of antitumor monoclonal antibodies by mechanism of action on target cells

III Monoclonal antibodies targeting ligands that inhibit T-lymphocyte activity (PD-1/PD-L1) or target costimulatory molecules (CTLA-4)

1. Monoclonal antibodies specific to PD1 (programmed cell death protein-1)

Nivolumab*, Pembrolizumab*

2. Monoclonal antibodies specific to PD-L1 (PD-L1 programmed cell death ligands)

Atezolizumab*, Avelumab*, Durvalumab*

3. Monoclonal antibodies specific to CTLA-4 **(cytotoxic T-lymphocyte-associated protein 4)**

Ipilimumab*

Monoclonal antibodies specific to antigens expressed on tumor cells (to CD20 protein)

Rituximab (Mabthera) - chimeric MAbs with specificity to the CD20 receptor, which is expressed on the surface of pre-B-lymphocytes and mature B-lymphocytes.

Obinutuzumab – humanized MAbs, selectively interacts with the extracellular site of the transmembrane AG CD20 located on the surface of normal and malignant MAbure B-lymphocytes and their precursors

Ofatumumab – a human MAb that specifically binds to an epitope that includes extracellular loops of the CD20 molecule, induces complement binding and activation, leading to the development of CPCs and tumor cell lysis. Ofatumumab induces lysis of rituximab-resistant cells.

Mechanism of antineoplastic action:

-cell-dependent cytotoxicity

-antibody-dependent cytotoxicity

Application: Treatment of non-Hodgkin's lymphoma, follicular lymphoma, chronic lympholeukemia.

Monoclonal antibodies that block Epidermal Growth Factor Receptor (EGFR) activity.

Normal cells cannot proliferate without the external influence of endogenous mediators (**growth hormones, cytokines, growth factors, etc**.), which are produced by cells of a certain phenotype. Growth factors induce proliferation through their interaction with appropriate receptors present on the cell membrane. Tumor cells are characterized by hyperexpression of receptors to growth factors, and they not only actively respond to growth factors, but also have the ability to synthesize them independently.

- **Cetuximab (Erbitux) -** chimeric MAbs specific to **EGFR,** block the binding of growth factors to the receptor, suppressing their functions, which leads to inhibition of proliferation and induction of apoptosis of tumor cells. In addition, cetuximab suppresses the production of factors stimulating angiogenesis by tumor cells and migration of epithelial cells.
- **Application** - metastatic colorectal cancer, non-small cell lung cancer

Monoclonal antibodies specific to the HER2 receptor

The **Her-2/ neu** receptor belongs to the family of type II EGFR receptors, which is present in tissues and in normal tissues, participating in the regulation of cell division and differentiation. In tumorigenesis, the expression level increases significantly (in breast cancer, gastric adenocarcinoma and lung cancer).

- **Trastuzumab (Herceptin)** - humanized MAb (IgG1), selectively interacts with HER-2 receptor expressed on tumor cells, suppresses proliferation of tumor cells, which are characterized by HER2 overexpression, through complement-dependent cytotoxicity (CDC).
- **Application** - breast cancer with tumorigenic HER2 overexpression, advanced gastric cancer.
- **Pertuzumab** – humanized MAbs, inhibit intracellular signaling through two major signaling pathways, the mitogen-activated protein kinase (MAP) pathway and the phosphoinositide-3-kinase (PI3K) pathway, leading to suppression of tumor cell proliferation and activation of the apoptosis mechanism. Pertuzumab also induces tumor cell death by activating the ADCC mechanism.
- **Application:** in cases of metastatic breast cancer in the form of monotherapy. The drug is more effective when combined with trastuzumab.

Monoclonal antibodies blocking vascular endothelial growth factor activity.

Many malignant tumors secrete factors that provide angiogenesis, such as **vascular endothelial growth factor (VEGF)**, which has mitogenic, chemotactic properties, so blocking this factor can inhibit tumor growth

Bevacizumab (Avastin) - humanized MAbs (IgG1) specific to VEGF. Suppresses tumor tissue angiogenesis and tumor metastasis due to binding of VEGF to its receptors on the surface of endothelial cells, enhances antitumor effect of a number of cytostatics.
Application – in metastatic colorectal cancer, breast cancer, lung cancer, renal cell cancer, glioblastoma, epithelial ovarian cancer, cervical cancer.

Aflibercept is a recombinant hybrid protein specific to VEGF.
It suppresses the formation of new blood vessels that help supply tumor tissue with oxygen and nutrients that stimulate tumor growth.
Application - metastatic colorectal cancer as monotherapy

Monoclonal antibodies targeting ligands that inhibit *T-lymphocyte activity (PD-1/PD-L1)* or target *costimulatory molecules (CTLA-4)* (immune checkpoint inhibitors)

- The physiological role of **the CTLA-4 and PD-1** signaling pathways is to prevent the development of a strong immune response.
- **The cytotoxic T-lymphocyte-associated protein CTLA-4** regulates the activation of T-lymphocytes in lymphoid tissues, where the immune response is initiated, and **the programmed cell death-1 protein PD-1** limits the activity of effector T-lymphocytes in tissues in the periphery, exercising control over the realization of the immune response
- The antitumor effect of MAb drugs - **checkpoint inhibitors** is based on the blockade of CTLA-4 and PD-1/PD-L1 signaling pathways controlling different stages of immune response.

Monoclonal antibodies specific to CTLA-4.

- **Ipilimumab** - human MAb **specific to CTLA-4 AG expressed on T-LF**, promotes proliferation and MAburation of antitumor cytotoxic T-LF, activation of effector mechanisms of antitumor immunity and tumor cell death.
- **Application**: metastatic melanoma in case of ineffectiveness of previous therapy.

Monoclonal antibodies specific to PD-L1 and PD1.

Nivolumab – a human MkAT directed to the PD-1 receptor expressed on T cells, blocks its interaction with **PD-L1 and PD-L2 ligand** presented on tumor cells

- prevents the death of T cells in the body
- activates antitumor immune response
- inhibits the growth of malignant tumor

Application - treatment of melanoma in case of its metastasis

Pembrolizumab - humanized high-affinity MAb, **highly selective blocker of PD-1** receptors expressed on T-cells, prevents suppression of antitumor immunity by limiting the escape pathways of tumor cells from the immune system's surveillance

Application - treatment of late-stage melanoma, metastatic non-small cell lung cancer, anaplastic lymphoma

Avelumab – (target: programmed cell death ligand 1 (PD L1)) for the treatment of lung cancer, breast cancer, hepatocellular carcinoma, melanoma.

Conjugated MAb

Depending on the **active substa**nce attached to the antibody, conjugated MAbs are categorized into the following groups:

- ***with radioactive particles (radioimmunotherapy)***
- **Ibritumomab tiuxetan*, Ibritumomab tiuxetan***
- ***with cytostatic* - anthracyclines (doxorubicin, daunorubicin, epirubicin), alkaloids, mitomycin-C**
- ***with toxins (or immunotoxins)***

Trastuzumab emtansine*, Brentuximab*, Gemtuzumab ozogamicin*, Gemtuzumab ozogamicin*

Due to MAb, the drug specifically binds to tumor cells expressing the corresponding antigens, exerting a dual effect on them by activating the mechanisms of AZCC and CPC, as well as the action of a radioactive isotope or other antitumor component.

Antibody-toxin (or immunotoxin) conjugates

Antibody-toxin conjugates are **MAbs coupled to cytotoxic agents**. The main cytotoxins are microtubule inhibitors or chemotherapeutic agents that damage DNA.

Trastuzumab emtansine* (Cadsila) is a conjugate of humanized MAb specific to the human HER2 receptor (trastuzumab) and the tubulin polymerization inhibitor DM1 (meitansin derivative), which has a cytotoxic effect.

Trastuzumab emtansine provides **specificity** of interaction with HER2 and delivery of DM1 inside tumor cells with hyperexpression of the indicated receptor.

DM1 causes cell cycle blockade in the G2/M phase and apoptosis of tumor cells. When the drug binds to the receptor, it inhibits intracellular signal transmission through the phosphatidylinositol-3-kinase (PI3-K) pathway, which contributes to the activation of AZCC and CPC, causing tumor cell death.

Application - in metastatic breast cancer.

Brentuximab* – (Adcetris) is a conjugate of MAb to CD30 and the antitumor agent monomethyleuristatin E for the treatment of lymphoma.

Antibody-toxin conjugates (or immunotoxins)

Immunotoxins are prepared by attaching bacterial (diphtheria toxin, Pseudomonas exotoxin) or plant toxins (ricin A or saporin) to MAb.

Gemtuzumab ozogamicin* (Mylotarg) - human antibodies to the CD33 antigen, which is present on most leukemic cells, in combination with the DNA-damaging toxin calicheamicin. Application - therapy for acute myeloblastic leukemia in the elderly.

Inotuzumab ozogamicin* (InO/CMC-544) is a humanized antibody-drug conjugate directed against CD22 bound to calicheamicin. The compound has been approved by the FDA for use in relapsed or refractory **acute lymphoblastic leukemia**

Conjugates of monoclonal antibodies with radioactive particles (radioimmunoconjugates)

When developing radioimmunoconjugated drugs, MAbs with short half-lives (murine) are used. HeMAbologic tumors (lymphoma, leukemia) are more sensitive to radiation compared to solid tumors.

Ibritumomab tiuxetan* (Zevalin) is a radioactive isotope AT conjugate that consists of recombinant murine MAb specific to the B-cell AG CD20 and the radioactive isotope yttrium-90. By specifically binding to CD20-expressing tumor cells, yttrium-90-labeled MAb has a dual effect on them by activating the mechanisms of AZCC and CPC, as well as the action of the radioactive isotope. Application: follicular B-cell lymphoma

Tositumomab* (Bexar) - mouse MAbs to the CD20 antigen to which the radioactive isotope iodine-131 is attached. The drug received FDA approval in 2003 for the treatment of relapsed follicular lymphomas. The drug is not registered in Russia

Monoclonal antibody conjugates with antitumor agents

- **Anthracyclines (doxorubicin, daunorubicin, epirubicin), alkaloids, mitomycin-C** can be used as antitumor agents in conjugated MAb drugs.
- Currently, **no drug formulations of MAb conjugated with chemotherapeutic agents have been registered**, and research on their development is ongoing.
- The most promising are **bispecific** antibodies that bind two molecular targets at once, which increases the recognition of malignant cells. In any case, the use of MkAt in the therapy of malignant neoplasms is a new and highly promising direction in modern oncology.

Modified MAbs

• **Blinatumomab** is a bispecific AT consisting of antigen-binding domains of two MAbs, one of which is specific to the CD19 receptor expressed on tumor cells, the other to CD3 expressed on T-lymphocytes.
The structure of such modified MAb, **allows binding and convergence of the lymphoid tumor cell and cytotoxic T-Lf.**

• **Application**: B-cell **acute lymphoblastic leukemia.**

Monoclonal antibodies in modern rheumatology

The main targets are cytokines and their receptors, membrane molecules of lymphocytes.

Classification of monoclonal antibodies depending on the point of application

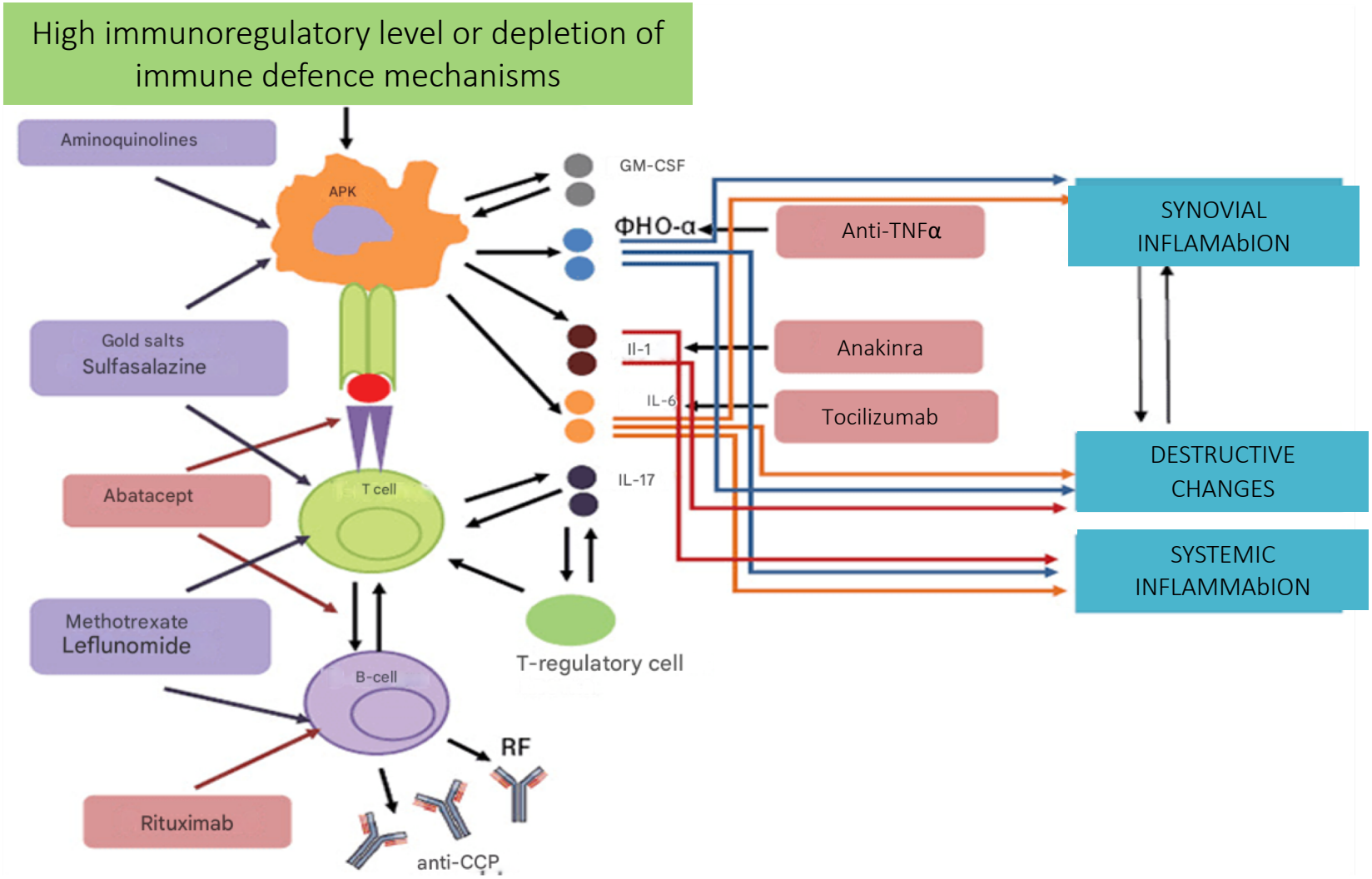
1. TNF (tumor necrosis factor) inhibitors – infliximab*, etanercept*, certolizumab, golimumab, adalimumab*

2. Interleukin receptor blockers – tocilizumab* (IL-6R), sarilumab* (IL-6), canakinumab* (IL-1R), secukinumab* (IL-17R)

3. Anti-B-cell antibodies (antibodies to CD20 membrane molecules) are rituximab*, belimumab*, ocrelizumab*

4. Anti-T-cell antibodies (antibodies to CD80 and CD86 molecules) – abatacept*

Pathophysiologic targets of monoclonal antibodies are interleukins, TNF, and lymphocyte surface proteins.



TNF inhibitors

• **Tumor necrosis factor inhibitors -** bind to TNF-a, preventing its interaction with specific receptors. TNF-a is an endogenous cytokine that participates in the immune response, in the development of pathological inflammation and destruction of joint tissue, characteristic of rheumatoid arthritis and other autoimmune diseases

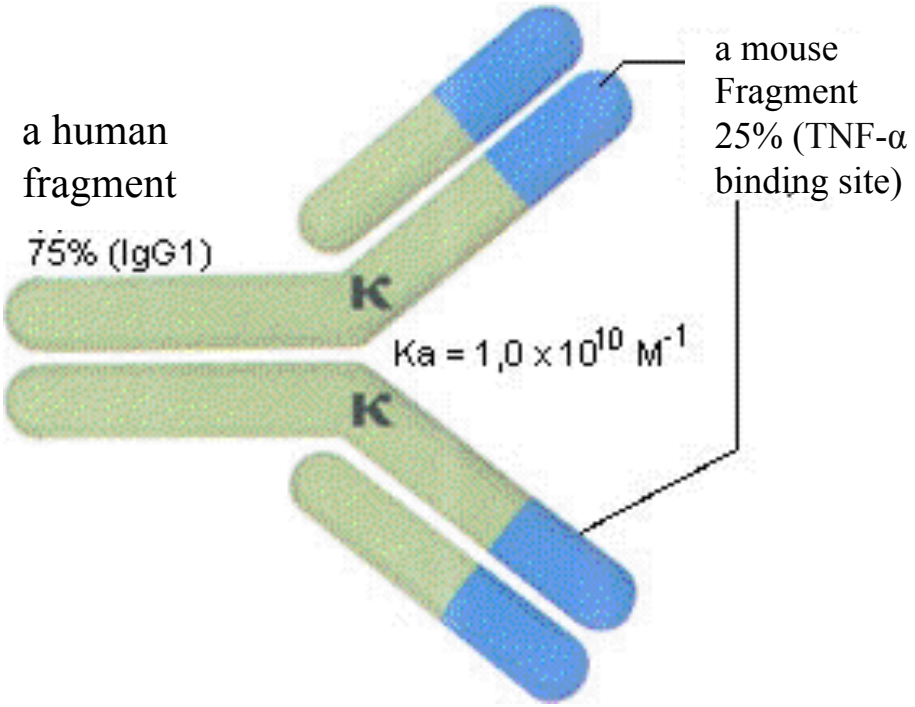
Infliximab (Remicade) is a chimeric MAb with high specificity that:

-binds and neutralizes transmembrane and soluble TNF-α in liquid medium;

-induces lysis of TNF- producing cells by complement fixation or by (AZCC);

- inhibits the biological activity of IL-6 in RA;

- inhibits proliferation of activated B-lymphocytes and synthesis of immunoglobulins



TNF inhibitors

- **Adalimumab (Humira) -** human recombinant monoclonal antibodies to TNF-a. Effective for the treatment of rheumatoid arthritis resistant to standard therapy.

- **Etarnecept (Enbrel)** - recombinant protein drug, which is produced by combining TNF-receptors with the Fc- fragment of the IgG1 molecule, blocks excess TNF by binding and inactivating TNF-α molecules.

Interleukin receptor blockers

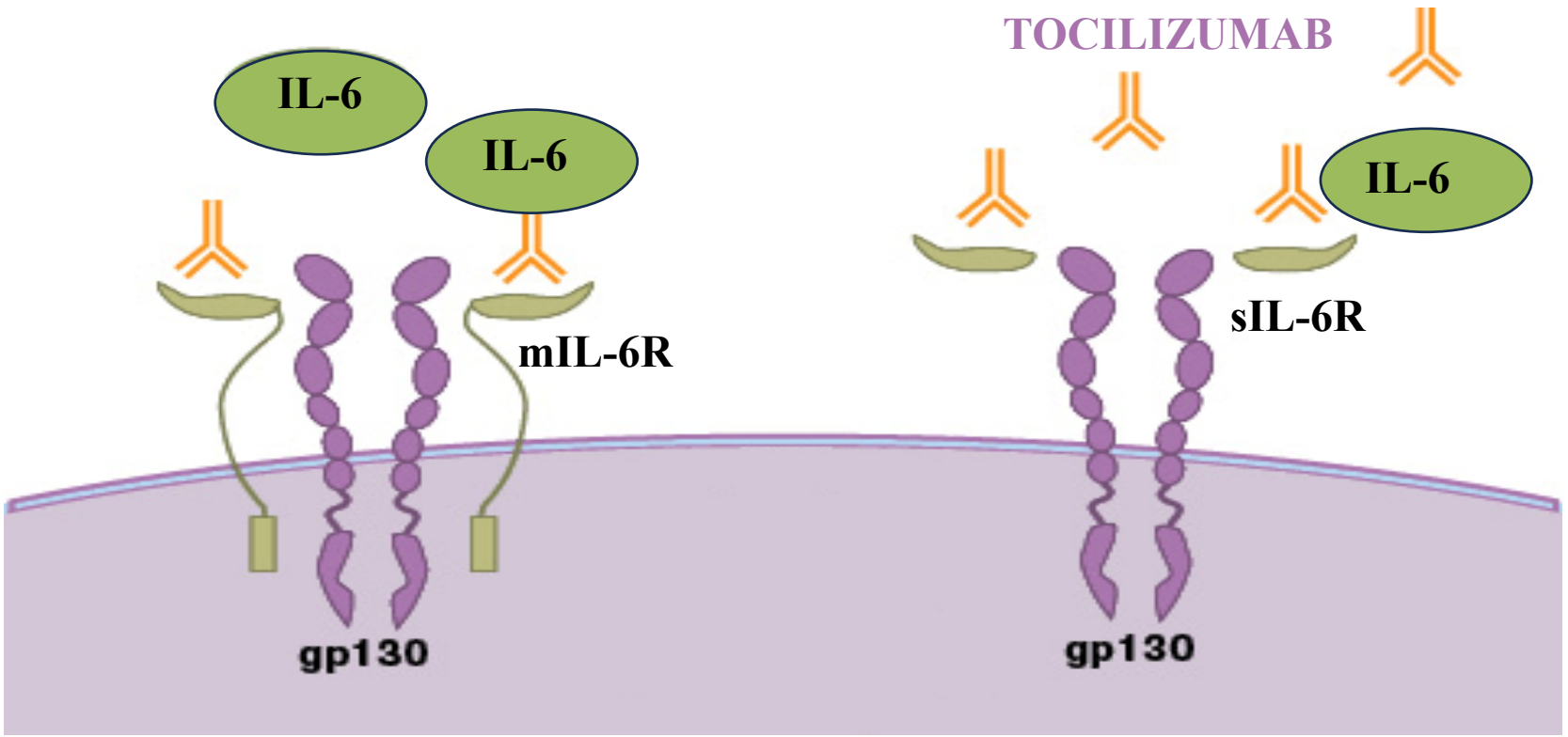
Interleukins (pro-inflammatory cytokines) play an important role in the development of autoimmune inflammation. The function of **interleukins** is to control the processes of differentiation, proliferation and death (apoptosis) of immune cells, which is realized through the corresponding target genes.

IL-6 is a multifunctional cytokine involved in paracrine regulation, systemic physiological and pathological processes, stimulates Ig secretion, activates T-cells, stimulates the production of acute phase proteins in the liver, and hemopoiesis. **IL-6 is involved in the pathogenesis of various autoimmune, inflammatory diseases, osteoporosis and neoplasms.**

Interleukin receptor blockers

Tocilizumab is a recombinant humanized monoclonal antibody to the human interleukin-6 (IL-6) receptor from the IgG1 immunoglobulin subclass.

Mechanism of action - selectively binds soluble and membrane IL-6 receptors, inhibiting both signaling pathways of IL6-dependent cell activation



Interleukin receptor blockers

- **Anakinra (Kineret**) – a recombinant soluble soluble competitive IL-1 receptor inhibitor that suppresses functional IL-1 activity.
- **Sarilumab**, levilumab - recombinant human monoclonal antibodies against interleukin-6 receptor (IL-6R), binds specifically to both soluble and membrane-bound IL-6 receptors
- **Siltuximab**, a chimeric monoclonal antibody that binds to interleukin-6 (IL-6), inhibits IL-6-mediated B-lymphocyte and plasma cell growth, vascular endothelial growth factor (VEGF) secretion, and autoimmunity
- **Application** - rheumatoid arthritis and other autoimmune diseases (psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis)

Anti-B-cell therapy

B-cells synthesizing rheumatoid factors have the ability to interact with immune complexes and “present” a wide range of autoantigens, and activated B -cells express co-stimulatory molecules.

CD20 is a B- cell surface antigen (phosphoprotein) that is expressed only by pre -B- and mature B -cells. CD20 is not present in stem cells and in plasma cells that produce immunoglobulins. This feature makes the CD20 protein an ideal “target” for biological drugs. When its activity is turned off, neither the formation of new lymphocytes nor the production of normal antibodies is impaired.

Anti-B-cell therapy

Rituximab - chimeric anti-CD20 monoclonal antibodies

Ocrelizumab (OCR, Ocrelizumab) - humanized antibodies to CD20 B cells, selectively bind and remove CD20+ B cells. This triggers immunologic reactions against B-lymphocytes that provide destruction (lysis) of these cells.

Mechanism of action of rituximab, ocrelizumab

1.Antibody-dependent cellular cytotoxicity, in which natural killer cells participate, attaching with the help of their Fc-receptors to the antititle on the surface of CD20+ B-cells, which induces the lysis of CD20+ B-cells, as well as monocytes and macrophages.

2. Complement-dependent cellular cytotoxicity, in which the PT - CD20 immune complex formed binds the C1q subcomponent of C1 protein, which leads to complement activation and forMAbion of a membrane-attacking complex and ultiMAbely induces CD20+ B-cell lysis

3. Stimulation of CD20+ B-cell apoptosis.

Ocrelizumab induces AZCC more pronounced than CPCs of target cells, due to this it causes less pronounced undesirable effects (primarily infusion reactions) than RT

Anti-T-cell therapy

In patients with rheumatoid arthritis, T-lymphocytes are found in the synovial fluid. Full activation of T-lymphocytes requires **2 signals** from antigen-presenting cells: **the first** is for recognition of specific antigen by T-cell receptors; **the second** (non-specific) co-stimulatory signal involves binding of CD80 and CD86 molecules on the surface of antigen-presenting cells to the CD28 receptor on the surface of T-lymphocytes. The combination of these interactions causes activation of T cells, which stimulate the production of pro-inflammatory cytokines.

Abatacept is a hybrid soluble protein consisting of the extracellular domain of cytotoxic T lymphocyte antigen-4 (CTLA-4) bound to a modified Fc fragment of human IgG1.

- selectively modulates a key co-stimulatory signal required for full activation of T lymphocytes,

- binds CD80 and CD86 proteins on the surface of antigen-presenting cells

- reduces T-lymphocyte-dependent antibody formation and inflammation

Monoclonal antibodies and SARS-CoV-2

- Monoclonal antibody therapy significantly reduces the risk of severe disease or death due to SARS-CoV-2 infection
- One therapeutic approach is **to target the SARS-CoV-2 spike protein (S),** which is responsible for coronavirus entry into host cells by binding to the angiotensin-converting enzymee 2 (ACE2) receptor

Classification of MAb-based drugs for treatment and prevention of COVID-19 by mechanism of action

1. Drugs for treatment and prophylaxis of COVID-19 based on MAb specific to SARS- CoV-2 (viral neutralizing MAb)

single-component **- sotrovimab, regdanvimab, bamlanivimab.**

combined **- bamlanivimab + etesevimab; casirivimab + imdevimab,**

tixagevimab and cilgavimab.

2. Non-SARS-CoV-2 virus-specific MAb-based drugs for the treatment of COVID-19

IL-6 receptor blockers

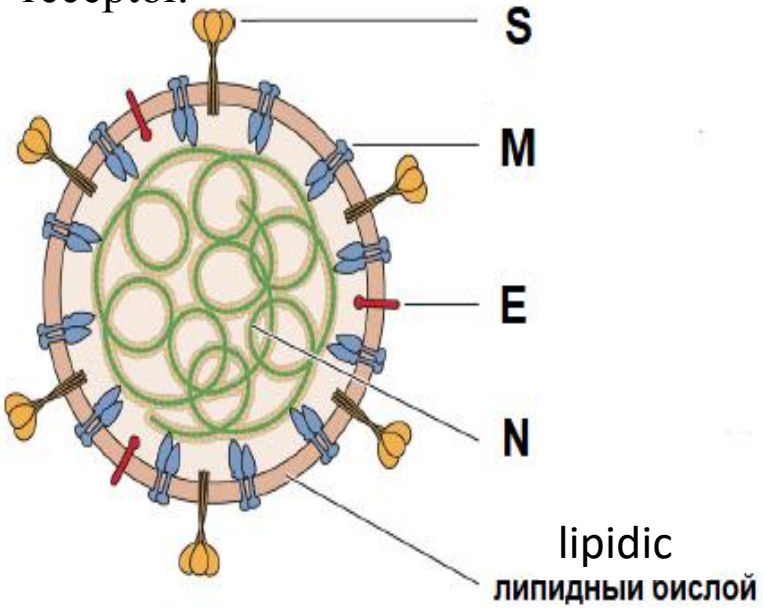
Tocilizumab* (atlizumab, Actemra**), sarilumab*, siltuximab, levilimumab*** **(**Ilsira**), olokizumab* (**Artlegia**)**

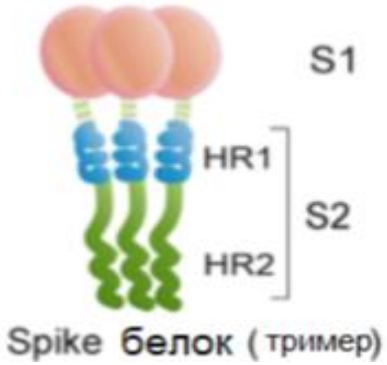
Monoclonal antibodies against CD6

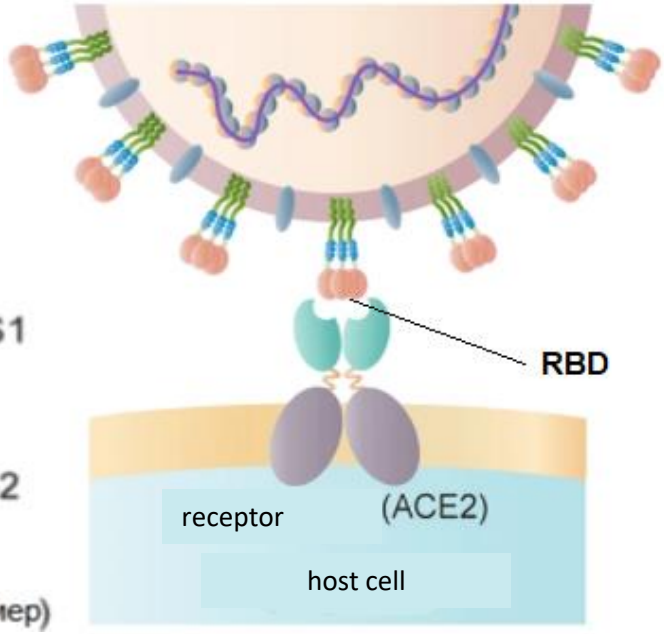
Itolizumab (Alzumab**)**

Mechanism of action of neutralizing MAbs for the treatment and prevention of COVID-19

The target for all MAbs neutralizing SARS- CoV-2 is the virus spike **glycoprotein (S-protein),** which gives SARS-CoV-2 the appearance of a "virus with a crown" and ensures virus entry into the human cell. It is a transmembrane homotrimer and has 2 functional subunits that mediate virus attachment to the host cell. The S1 subunit is formed by 4 domains, **the most important of which are the N-terminal domain (NTD)** and the receptor binding domain. Binding of S-protein MAb neutralizes **SARS-CoV-2 entry into the cell by blocking epitopes** that are involved in binding of the virus to the ACE2 receptor.

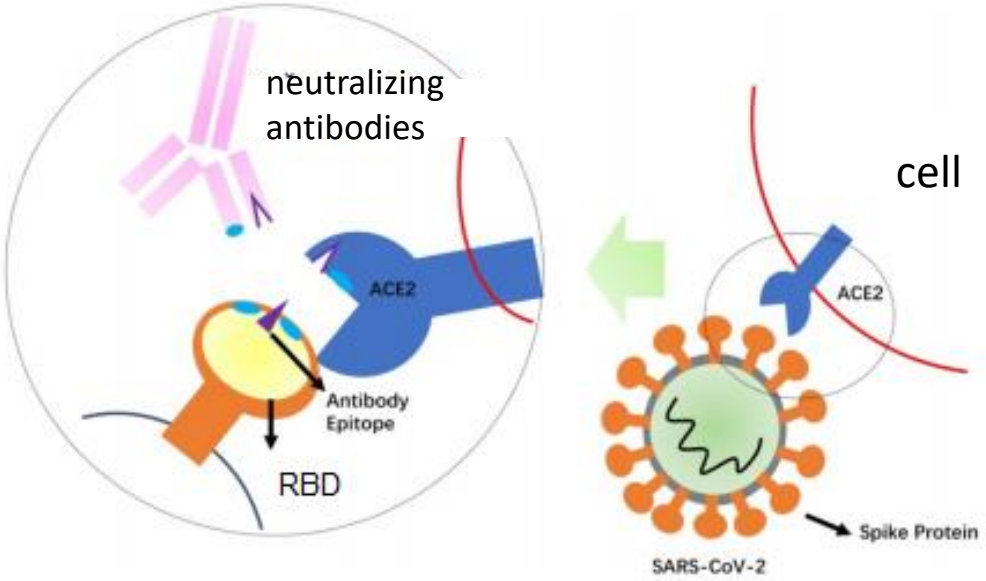






(trimer)

Mechanism of action of neutralizing MAbs for the treatment and prevention of COVID-19



- bind to non-overlapping epitopes of the receptor-binding domain (RBD) of S-protein SARS-CoV-2
- block the interaction of the S-protein SARS-CoV-2 with angiotensin-converting enzyme 2 (ACE2)
- neutralize the entry of SARS-CoV-2 into the cell
- stop viral replication

Mechanism of action of IL-6 receptor blockers for the treatment of COVID-19

- The immune response in severe COVID-19 can provoke a sudden release of large amounts of pro-inflammatory cytokines (including IL-6) into the bloodstream, accompanied by the development of **cytokine storm syndrome** and a life-threatening systemic inflammatory response. Elevated IL-6 concentrations in patients' blood correlate with severe complications in COVID-19.
- **IL-6 receptor blockers**

- bind and block both soluble and membrane IL-6 receptors

- prevent the development of an IL-6-associated pro-inflammatory cascade

- prevent activation of antigen-presenting cells - B- and T-lymphocytes, monocytes and macrophages, endothelial cells, fibroblasts, excessive production of other pro-inflammatory cytokines

- **FDA** approved them for the prevention of complications associated with COVID-19-induced cytokine storm.

Mechanism of action of anti-CD6 monoclonal antibodies for the treatment of COVID-19

Anti-CD6 monoclonal antibodies also work to prevent cytokine storm by targeting specific molecules on the cell surface that are involved in the regulation of the immune response.

Itolizumab (Alzumab) – a humanized IgG1 monoclonal antibody

- selectively binds on **CD6**, which is involved in T-cell co-stimulation, adhesion, and maturation

- reduces regulation of T-cell activation

- causes a decrease in the synthesis of pro-inflammatory cytokines

-reduces T-cell infiltration at sites of inflammation

In India, the use of itolizumab has been approved to prevent the development of “cytokine storm” in patients with acute respiratory distress syndrome of moderate and severe severity on the background of COVID-19.

Classification of MAb-based drugs for the prevention and treatment of COVID-19 by clinical use

1. **Drugs for pre-exposure prophylaxis of COVID-19 infection MAb**

combination of **tixagevimab and cilgavimab (Evushheld**)

2. MAb drugs for post-exposure prophylaxis and treatment of COVID-19 infection

MAb combination **casirivimab + imdevimab (REGN-COV2)**

MAb combination **bamlanivimab + etesevimab**

MAb combination **tixagervimab and cilgavimab (Evushheld**).

3. MAb drugs, for the treatment of COVID-19

sotrovimab, bebtelovimab

bamlanivimab + etesevimab, casirivimab + imdevimab MAb combination

MAb drugs for pre-exposure prophylaxis COVID-19

- **Tixagevimab and cilgavimab** are MAb-based drugs with a mutation-modified Fc-fragment. Modification of the Fc-fragment allows prolonging the half-life of the drug when administered intramuscularly. The drug implies protection from infection for up to 6-12 months.
- **Application:** intended for persons who are not yet infected with SARS-CoV-2 and have not had recent contact with infected SARS-CoV-2) as an alternative to vaccines for persons who have contraindications for vaccination, recommended for persons who are on treatment with immunosuppressants.
- **MAb drugs for post-exposure prophylaxis and treatment of COVID-19**
- **Casirivimab and imdevimab** are neutralizing human MAb specific to SARS-CoV-2.
- **Application:**

- for the treatment of mild to moderate COVID-19 patients who are at high risk for disease progression

- for post-exposure prophylaxis of COVID-19 in patients exposed to SARS-CoV-2 who are at high risk for progression to severe COVID-19 (individuals who cannot be vaccinated, immunodeficient

MAb drugs for COVID-19 treatment

- **Bamlanivimab, regdanvimab, sotrovimab**
- **The combination of bamlanivimab with etesevimab** is an S-protein neutralizing MAb that was developed from the original specific immunoglobulins produced by B-lymphocytes derived from two separate patients who had survived COVID-19. Combining these 2 neutralizing MAbs in clinical use may help to reduce viral load and decrease the likelihood of resistant variants emerging with treatment
- **Sotrovimab** is an Fc-fragment-modified MAb. Sotrovimab has a **2 amino acid substitution in the Fc-fragment domain.** This modification is made to increase the half-life while preserving effector function
- **Regdanvimab** - recombinant human monoclonal antibody, immunoglobulin G1, inhibitor of viral internalization, targeting SARS-CoV-2 spike protein.
- **Indications -** for the treatment of mild to moderate disease in COVID-19 positive patients at high risk of progression to severe disease.