VOLGOGRAD STATE MEDICAL UNIVERSITY
DEPARTMENT OF PHARMACOLOGY AND BIOINFORMATICS

Methodological recommendations for teachers for practical classes in the discipline:
"Immunobiologic and genotherapeutic medicines"

Lesson topic:
Recombinant drugs: Monoclonal antibodies in rheumatology, transplantology, oncology and oncohematology, in the treatment of COVID-19.

Faculty of Pharmacy

1. OBJECTIVES OF THE LESSON.

- to learn to analyze the effect of recombinant drugs (monoclonal antibodies) used in rheumatology, transplantology, oncology and oncohematology, in the treatment of COVID-19 according to the totality of their pharmacological properties, mechanisms and localization of action;
- to learn to evaluate the possibilities of using recombinant drug pre-drugs (monoclonal antibodies) for therapy of oncologic, autoimmune, viral diseases (COVID-19), for prevention and treatment of transplant rejection reaction;
- to learn to evaluate possible side and toxic effects of this group of immunobiologic drugs;
- to learn about the necessity of educating the population on the effectiveness of monoclonal antibodies for the therapy of oncological, autoimmune, viral diseases (COVID-19), for the prevention and treatment of graft rejection reactions - as a significant factor in the fight against severe, chronic diseases;

2. OBJECTIVES.

For recombinant monoclonal antibody-based drugs used in oncology, rheumatology, for treatment and prevention of COVID-19 virus infection and transplant rejection reaction study:

- Classifications;
- general characterization and mechanism of action;
- basic mechanisms of action and clinical targets;
- side effects of drugs;
- application in medicine, immunodiagnostics

3. THE FOLLOWING PRACTICAL SKILLS AND ABILITIES WILL BE TRAINED IN THE CLASSES

- ability to classify drugs of the studied groups depending on their mechanism of action and clinical use;
- ability to identify the type of monoclonal antibody by the name of recombinant therapeutic drug and assess its immunogenicity;
- ability to evaluate the possibilities of recombinant drugs (monoclonal antibodies) application in oncology, rheumatology, for treatment and prevention of viral infection COVID-19 and transplant rejection reaction;
- ability to analyze possible side and toxic effects of recombinant drugs (monoclonal antibodies);

4. ORDER OF CLASSES:

Venue: classroom of the Department of Pharmacology and bioinformatics. Time: part 1 – 2 academic hours

Formed competencies: UK-1.1.3, UK-1.2.1, UK-1.2.2, UK-1.2.3., UK-1.3.1, UK-1.3.2., UK-6.1.1., UK-6.2.1, UK-6.2.2, UK-6.3.1, UK-6.3.2, УUK-6.3.3, УKU-6.3.4, OPK-1.1.1., OPK-1.2.1, OPK-1.2.2., OPK-1.3.1, OPK-6.1.1, OPK-6.2.1, OPK-6.3.1, PK-7.1.1, PK-7.2.1, PK-7.3.1.

4.1 Technological map of the lesson

Part	№	Lesson stage	Time
1	1	Checking the students present in the lesson, mode of the lesson, topic of the lesson.	5 minutes
	2	Checking the initial level of knowledge of students (written survey).	10 minutes
	3	Survey on the topic of the lesson.	45 minutes
	4	Independent work of students (on prescriptions with analysis of the most complex recipes (if any in the topic), analysis of errors in medical prescriptions written by students; work with synonyms).	15 minutes
	5	Checking independent work	5 minutes
	6	Summing up the lesson. Assignment for the next lesson.	5 minutes
	7	Cleaning workplaces.	5 minutes

4.2 Demonstrations

1. Demonstration of advertising brochures on this topic during a survey on the topic of the lesson.

4.3 Lesson plan

4.3.1 The lesson begins with an introductory speech from the teacher, stating the purpose of the lesson and answering students' questions.

The importance of the topic in the system of training and activities of the pharmacist:

- informing the population on the effectiveness of the use of monoclonal antibodies in oncology, rheumatology, for the treatment and prevention of the COVID-19 viral infection and transplant rejection.

4.3.2 Checking the initial level of knowledge of students (written survey).

4.3.3 Analysis of theoretical material.

Plan for analysis of theoretical material

I Application of monoclonal antibodies in oncology and oncohematology

Two types of MABs are used in tumor therapy:

1. 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

2. 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.

In immunotherapy of malignant neoplasms, unconjugated MAbs that bind to receptors on tumor cells and cause their death through immune and non-immune mechanisms are more often used.

Mechanisms of antitumor action of therapeutic monoclonal antibodies

1. **Immune mechanisms**

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

Monoclonal antibodies can activate AZCCs by recognizing their Fc-domain by receptors located on the surface of immune effector cells (primarily natural killer cells). The effector cells recognize antibody-coated tumor cells and cause their lysis

Complement-dependent cytotoxicity (CDC)

In CPC, a multistep proteolytic cascade is formed, resulting in the death of foreign cells due to the assembly of a membrane-associated complex. The antibody binds to the antigen on the surface of the target cell, which leads to the activation of the complement system by the classical pathway. The formation of membrane-associated complex and subsequent release of anaphylins and opsonins leads to lysis and phagocytosis of target cells

- Antibody-dependent phagocytosis

Macrophages (and monocytes) can also phagocytose tumor cells in the presence of MAB. AZP 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 a cytotoxic lymphocyte, the so-called "killer". The latter synthesize and secrete proteins perforins and serine proteases that damage the cell membrane, causing its lysis.

2. Non-immune mechanisms

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

Some malignant cells have a large number of receptors for growth factors on their surface, which activate a cascade of reactions aimed at enhancing cell proliferation. If a receptor is blocked with a monoclonal antibody, it cannot bind to the ligand (growth factor) and the cascade of these reactions will not be triggered, leading to cell death.

- induction of apoptosis

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

1.1. Classification of antitumor monoclonal antibodies according to the 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 HER2 receptor.
Trastuzumab, Pertuzumab

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

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

1.3. Pharmacological characteristics of monoclonal antibodies specific to antigens expressed on tumor cells (to the CD20 protein), mechanisms of action, application.

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 mature B-lymphocytes and their precursors

Ofatumumab – a human MAbs 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.

1.4. Pharmacological characteristics of monoclonal antibodies blocking the activity of the epidermal growth factor EGFR (Epidermal Growth Factor Receptor), mechanisms of action, application.

- **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

- **Panitumumumab** is a human MAb (IgG2) characterized by high affinity and specificity for human EGFR receptors. Binding of panitumumumab to EPR leads to inhibition of autophosphorylation, suppression of cell growth processes, induction of apoptosis and reduction of IL-8 and vascular endothelial growth factor production.
- **It is used as monotherapy** in metastatic colorectal cancer with REFR expression after the previous course of chemotherapy.

1.5. Pharmacological characteristics of monoclonal antibodies specific to the HER2 receptor, mechanisms of action, application.

The **Her-2/ neu** receptor belongs to the family of type II EPR 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.

1.6. 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.

1.7. 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.

Ipilimumab - human MAb **specific to CTLA-4 AG expressed on T-LF**, promotes proliferation and maturation 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.
Nivolumab – a human MAb 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

2. Conjugated MAbs

Depending on the **active substa**nce attached to the antibody, conjugated MATs are categorized into the following groups:
• with radioactive particles (*radioimmunotherapy*) – ibritumomab tiuxetan, tositumomab
• *with cytostatics – conjugation with anthracyclines, mitomycin C*
• *with toxins (or immunotoxins)* – trastuzumab emtansine, brentuximab, gemtuzumab ozogamicin, inotuzumab 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 ADCC and CDC, as well as the action of a radioactive isotope or other antitumor component.

2.1. Antibody toxin conjugates (or immunotoxins)

The main cytotoxins are microtubule inhibitors or chemotherapeutic agents that damage DNA.

Antibody-toxin conjugates are **MATs 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 **(IgG1)** 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.

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**

2.2. Conjugates of monoclonal antibodies with radioactive particles (radioimmunoconjugates)

When developing radioimmunoconjugated drugs, MATs with short half-lives (murine) are used. Hematologic 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 MATs 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

2.3. 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.

II 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.

2. Tumor necrosis factor 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 (ADCC), inhibits the biological activity of IL-6 in RA, inhibits proliferation of activated B-lymphocytes and synthesis of immunoglobulins

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.

3. 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

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)

4. 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.

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 formation of a membrane-attacking complex and ultimately 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 **rituximab**.

5. Anti-T-cell therapy

In patients with **rheumatoid arthritis (RA)**, 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** (nonspecific) costimulatory 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

III Use of MAbs in the treatment of COVID-19

1. Classification of drugs for the treatment and prevention of COVID-19 based on MAbs by mechanism of action

1. Drugs for the treatment and prevention of COVID-19 based on MAbs specific to SARS-CoV-2 (virus-neutralizing MAbs)
single-component - sotrovimab, regdanvimab, bamlanivimab
combined - bamlanivimab + etesevimab; casirivimab + imdevimab, tixagevimab and cilgavimab

2. Drugs for the treatment of COVID-19 based on mAbs not specific to the SARS-CoV-2 virus.
- IL-6 receptor blockers
Tocilizumab* (atlizumab, actemra), sarilumab*, siltuximab, levilimab* (ilsira), olo-kizumab* (artlegia)
- Monoclonal antibodies against CD6
Itolizumab (Alzumab)

1.2. 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.

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

1.2. 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 outcomes 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 proinflammatory cascade

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

1.3. 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 costimulation, adhesion, and maturation

- downregulates **T-cell activation**

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

-reduces T-cell infiltration at sites of inflammation

2. 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

2.1. MAb drugs for pre-exposure prophylaxis of COVID-19

Tixagevimab and cilgavimab are MAb-based drugs with an Fc fragment modified by mutations. Modification of the Fc fragment allows to extend the half-life of the drug when administered intramuscularly. The drug provides protection against infection for up to 6-12 months.

Application - intended for individuals who are not yet infected with SARS-CoV-2 and have not had recent contact with an infected SARS-CoV-2) as an alternative to vaccines for individuals who have contraindications for vaccination, recommended for individuals who are treated with immunosuppressants

2.2. 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

2.3. 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 MATs 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.

Both single-component and combination drugs are not registered in Russia. The procedure for prescribing monoclonal antibodies against the S-protein of coronavirus in Russia is fully regulated by the temporary guidelines for the prevention, diagnosis, and treatment of new coronavirus infection. According to them, MAb can be administered upon the decision of a medical committee.

IV Application of MAb in transplantology

Mechanisms of transplantation immunity.

The immune response to the transplant is due to the recognition of HLA antigens (human leukocyte antigens) and ABO antigens (may be the cause of organ rejection after transplantation) of the donor by the recipient's lymphocytes. This causes activation of T-helper cells, which stimulate proliferation of B-lymphocytes and cytotoxic T-lymphocytes.
Antibodies to foreign HLA antigens may be present in the recipient's serum before transplantation. Their detection indicates previous immunization with HLA antigens. This is possible with whole blood transfusion and during pregnancy. Detection of antibodies to HLA antigens of the donor in the recipient's serum indicates a high risk of superacute transplant rejection. It is caused by the formation of complexes consisting of graft antigens and recipient antibodies, which activate blood coagulation and lead to thrombosis of graft vessels.
Since graft rejection is caused by foreign HLA antigens, the best way to prevent it is to select a donor compatible with the recipient in terms of HLA antigens.

1.Anti-B- and T-cell antibodies

Muromonab-CD3 - mouse monoclonal antibodies against CD3 antigen located on the surface of T-lymphocytes, specifically reacts with CD3 glycoprotein, which is a part of antigen-recognizing receptor complex of T-lymphocytes, changes it, as a result of which the latter lose their immunological properties. Due to this interaction the reaction of transplant rejection is prevented.
Application: in acute rejection of allotransplants (kidneys, heart and liver), in homologous disease, in life-threatening situations when rejection is not controlled by steroids and anti-lymphocyte globulin.
Disadvantages: may develop cytokine release syndrome, completely alien to humans, repeated use is impossible.

Alemtuzumab is a humanized monoclonal antibody against CD52 antigen present on T- and B-lymphocytes, natural killer cells, monocytes and macrophages. After binding to the cell surface of T- and B-lymphocytes alemtuzumab leads to antibody-dependent cell cytolysis and complement-mediated lysis, rapid and persistent reduction in the number of T- and B-lymphocytes.
Application- in transplantology for introductory immunosuppression, which allows to provide maintenance immunosuppression without the use of GCS.
Side effects - lymphopenia, neutropenia, thrombocytopenia, diarrhea, swallowing disorders, nausea, vomiting, myalgia, infections, malignant tumors, hypotension, etc.

Rituximab - chimeric anti-CD20 monoclonal that blocks the proliferation of B- cells by ADCC, ADF, CDC.

Application - the drug is used as a desensitizing therapy for organ rejection, for ABO-incompatible transplantation, for treating rejection associated with B-cells and antibodies.

2.IL-2 receptor antagonists

Mechanism of action - inhibition of interleukin-2-mediated lymphocyte activation and inhibition of the immune reaction of transplant rejection.

Basiliximab is a chimeric MAb that specifically binds and blocks the α-subunit of the inter-leukin-2 receptor complex (IL- 2Rα, CD25 antigen) on the surface of activated T-lymphocytes and prevents interleukin-2 interaction with them. Prevents inter-leukin-2-mediated activation of lymphocytes and disrupts the immune system response to antigen. Does not cause cytokine release or myelosuppression.

Indications - prophylaxis of organ transplant rejection.

Daclizumab is a humanized MAb that binds highly specifically to the alpha-subunit of the high-affinity IL-2 receptor complex, which is expressed on activated T cells. Inhibits IL-2 mediated activation of lymphocytes, inhibits immune reaction of transplant rejection.

Side effects - anaphylactoid reactions, seizures, depression, anxiety, visual disturbances, arterial hypertension or hypotension, tachycardia, bleeding or thrombosis, cough, dyspnea, pulmonary edema, kidney damage, hydronephrosis, renal failure, fluid retention, dehydration, hematuria, urinary retention.

3.Inhibitors of the complement system

Eculizumab is a humanized monoclonal antibody that has anticomplement activity by blocking the terminal complement complex, binds specifically to protein C5, prevents the formation of the final complement link - the membrane-brane attacking complex.

Application - for desensitization in ABO incompatible kidney and pancreas transplantation, in the reaction of humoral rejection of the transplant, the key moment of which is the activation of the complement system with subsequent damage to the endothelium of the microcirculatory channel of the transplant.

4.3.5 Independent work of students:
1. Answer theoretical questions, including classification, mechanisms of action, pharmacological characteristics of MAb drugs used in oncology, oncohematology, rheumatology, transplantology, in the treatment of COVID-19. The information is recorded in students' workbooks.

2. Work with advertising brochures for medicines on this topic.

4.3.1 Checking students' independent work.
4.3.2 Summing up the lesson. Answers to students' questions.
4.3.3 Closing remarks from the teacher.

Compiler,
Associate Professor,
Ph.D. O.A. Salaznikova

Appendix 1

Перечень рекомендуемой литературы, включая электронные учебные издания:

1. Харкевич Д. А. Фармакология : учебник / Харкевич Д. А. - 11-е изд., испр. и доп. - М. : ГЭОТАР-Медиа, 2015. - 755, [5] с. : ил. - Текст: непосредственный.
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1. http://vrachirf.ru/ - Информационный портал Врачи России
2. https://pharmarf.ru – информационный портал Фарма России
3. https://www.rlsnet.ru/ - РЛС (регистр лекарственных средств России) (информационная справочная система)
4. http://www.drugs.com - Информационная база о лекарственных препаратах (информационная справочная система)
5. https://grls.pharm-portal.ru/ - государственный реестр лекарственных средств.
6. http://elibrary.ru – Электронная база, электронных версий периодических изданий на платформе Elibrary.ru (профессиональная база данных)
7. http://www.consultant.ru/ – Справочно-правовая система «Консультант-Плюс» (профессиональная база данных)