Immunobiological and gene therapy drugs. Classification, application in medicine. General requirements for the production of biological drugs. Immunobiological drugs.

Biological medicinal agent

- -medicinal products whose active substance is produced or isolated from biological material and for the determination of the properties and quality of which a combination of biological and physicochemical methods is necessary.

Classification of biotechnological drugs:

- biotechnological;
- immunological (vaccines, heterologous immunoglobulins and immunosera, diagnostics and allergens);
- plasma-derived drugs (albumin, coagulation factors, human immunoglobulin);
- advanced therapy drugs, including gene and cell therapy drugs, as well as tissue engineering drugs;
- non-recombinant drugs (botulinum toxins, digestive enzymes, urofollitropins, heparins, etc.).

Classification of biotechnological drugs:

- biotechnological drug are medicinal products manufactured using biotechnological processes and methods (including recombinant DNA technology, technology of controlled expression of genes encoding biologically active proteins in prokaryotes and eukaryotes, including modified mammalian cells), the hybridoma method and the monoclonal antibody method;
- Gene therapy drugs are drugs whose pharmaceutical substance is a recombinant nucleic acid or includes a recombinant nucleic acid that allows for the regulation, repair, replacement, addition or deletion of a genetic sequence.

Composition of biological drug

Biological medicinal may contain

- proteins, peptides and/or derivatives of proteins and peptides
- glycoproteins; live or inactivated microbes (bacteria, viruses), their antigens, antibodies to them, metabolites and other active substances of biological origin (for example, cytokines, monoclonal antibodies, cell receptors, recombinant proteins similar to cytokines or coagulation factors from blood plasma, vaccines based on recombinant proteins, etc.)
- auxiliary substances of various functional purposes (stabilizers, adjutants, sorbents, preservatives, fillers, etc.)

Features of biopreparations

- **high specificity of the mechanism of action** and “targeting”, which determines their low non-specific toxicity, and their unsafety is primarily mediated by the pharmacological action and immunogenicity, as well as the ability to uncontrolled replication or proliferation and growth;

- **high sensitivity to environmental factors**, as a result of which both production and storage, transportation, sale and use of biopreparations must be carried out in gentle, strictly controlled conditions. Producer cells are very demanding of maintenance conditions;
- **predominantly parenteral route of administra**tion (usually intravenously); since the drug enters the internal environment of the body, bypassing protective barriers, they must be carefully sterilized, which creates additional problems, since biopreparations are subject to biological contamination and cannot be subjected to aggressive sterilization and purification methods to eliminate the bioburden;
- **instability in the external environment**, which is why biopreparations, as a rule, must be in aqueous media or subjected to lyophilization, while low-molecular substances do not have such strong restrictions and can often be found in capsules, tablets, etc.;
- **the main focus is on developing the biological active ingredient**, ensuring homogeneity of its properties, purification and maintaining stability; developing the formulation (aqueous solution) is relatively simple compared to the efforts involved in developing the dosage form for low-molecular active ingredients (tablets, ointments, aerosols, patches).

Production of biological preparations

- The development of a biopreparation begins with the selection of a cellular target to which the drug is directed. Most often, this is a receptor on the surface of a living cell or another surface protein. After selecting a target, an antigen-binding site of an antibody of a certain amino acid sequence is selected for it (each antibody consists of an invariable part specific to a particular biological species - human, mouse, etc., and the antigen-binding region responsible for the action on a specific target) and tested in silico - undergoes computer modeling. Then, using the method of synthesis, the gene encoding this amino acid sequence is produced, with regions that ensure its activity in a living cell.

Methods of gene integration into the genome of a biomaterial cell

- Electroporation.

A short pulse of electric current is applied to the cell, causing the cell membrane to destabilize. The insert gene, built into a plasmid that functions as a vector – a DNA molecule – penetrates from the outside into the cell, integrating into the necessary parts of the chromosome. Why is it impossible to deliver a gene directly into the cell, bypassing the process of insertion into the vector?

As is known, a gene is a part of DNA, defined by a sequence of nucleic acids. And if this material gets inside the cell membrane, it will be dissolved without a trace by nucleatase enzymes, which are present in the cell cytoplasm. And this is where the study ends.

Methods of gene integration into the genome of a biomaterial cell

Viral vector.

The required gene is built into the protein structure of the neutralized virus. The virus then builds into the target cell, thus delivering the necessary material into the living cell. A virus-based vector is the most effective way to deliver genetic material into a cell. But it is very labor-intensive.

In order to begin inserting a gene into viral DNA or RNA, it is necessary to select a suitable virus that meets a number of parameters:

- Was stable, that is, not prone to spontaneous changes - mutations
- Did not affect the vital activity of the target cell
- Had a capacity to accommodate a large insert Integrated the genome into a specific location on the host chromosome
- Did not cause an immune response

Terms and definitions

adjuvant	a chemical or biological substance that enhances the immune response to an antigen and/or increases the duration of immunity;	antibodies	a chemical or biological substance that enhances the immune response to an antigen and/or increases the duration of immunity;
antigens	substances (e.g. toxins, foreign proteins, bacteria, viruses, tissue cells, etc.) that can cause specific immune reactions;	cell bank	a pool of homogeneous cells stored in portions in individual containers under specified conditions.

Terms and definitions

biological pharmaceutical substance	**a pharmaceutical substance produced using a biological source that must be characterized using physical, chemical and biological tests and whose quality is determined by these tests in combination with control of its production processes;**	**viral vector**	**a vector produced by modifying a virus using molecular biology techniques to retain some, but not all, of the virus's maternal genes. By removing the genes responsible for the virus's ability to replicate, the resulting vector is incapable of replication;**
vector	a transmission agent (a nucleic acid molecule, most often DNA) that carries genetic information from one cell or organism to another, such as plasmids, viruses;	excipients	substances of inorganic or organic origin used in the process of production and manufacture of medicinal products to give them the necessary physical and chemical properties1, except for pharmaceutical substances and packaging material;

Terms and definitions

gene	**a DNA sequence that codes for one or more proteins;**	**source materials**	**starting materials are any substances of biological origin, such as microorganisms, organs and tissues of animal origin, cells or fluids (including blood and plasma) of human or animal origin, as well as biotechnological cell substrates (recombinant and natural), including primary cells;**
rmain cell bank	a homogeneous suspension of host cells transformed with an expression vector. The suspension is poured into containers in equal volumes, frozen and stored under conditions that ensure their stability. The GBC is obtained under established conditions from a selected cell clone containing the expression construct;	cellular reserve	primary cells that have been expanded to a specified cell number, aliquoted, and used as starting material for the production of limited batches of cell-based medicinal products;

Terms and definitions

host cells	**microbial cells or eukaryotic cell lines used to obtain the product, prior to the introduction of the vector into them;**	**passage**	**transfer of a microorganism from one nutrient medium to another medium or from one host (animal, cell culture, etc.) to another;**
cell culture	cell mass obtained by growing in vitro cells isolated from multicellular organisms;	gene transfer	the process of transferring a gene into cells, including the expression system contained in a delivery system called a vector. The vector may be of viral or non-viral origin. After gene transfer, the genetically modified cells may also be called "transformed cells";

Terms and definitions

plasmid	**a piece of DNA that normally exists in a bacterial cell as a circular structure separate from the cell's chromosome. A plasmid can be modified using molecular biology techniques, isolated from a bacterial cell, and used to transfer and insert its DNA into the genome of another cell;**	**working cell bank**	**a homogeneous suspension of cells obtained at a given passage level by culturing cells from one or more GBC containers, dispensed in equal volumes into containers for storage under conditions that ensure their stability. RBC is used for the production of each batch of finished product. Samples of the working cell bank must be stored at least until the expiration date of the last batch of the product released;**
production strain	a strain of microorganisms that is stored in production under certain conditions and is used to prepare biological preparations;	producer strain	host cell-vector complex

Terms and definitions

specific pathogen free	**animal materials (e.g. chickens, embryos or cell cultures) used for the production or quality control of biological medicinal products, obtained from groups of animals (e.g. herds or flocks) free from specified pathogens. Such herds or flocks are defined as groups of animals that live in a common environment and have caregivers who are not in contact with animals that are not free from specified pathogens;**	**biosafety level**	**a set of measures and means to prevent contamination of the environment and personnel when working with biological agents of a certain pathogenicity group (I-IV);**

Terms and definitions

pure culture (axenic culture)	**a culture represented by microorganisms of only one species, characterized by common morphological, cultural, antigenic, biochemical and other properties)**	**expressive construction**	**a vector that contains the coding sequence of a recombinant protein and the elements necessary for its expression;**

General requirements for the production of BLS

- The production of biologically active drugs is characterized by the complexity and diversity of technological processes, since it is associated with biological processes and materials, such as cell cultivation or extraction of material from living organisms.
- Biological processes are characterized by variability, which leads to inconsistency in the spectrum and nature of by-products. Moreover, the materials used in the cultivation processes are themselves substrates for the growth of microorganisms.
- The safety of biological medicinal products is based on strict control of their starting materials. When assessing the risks of contamination of raw materials and starting materials, special attention is paid to the risk associated with contamination with pathogens of animal spongiform encephalopathy (OFS "Reducing the risk of transmission of pathogens of animal spongiform encephalopathy when using medicinal products") and latent viruses (OFS "Viral safety"). Attention should also be paid to source materials that come into direct contact with process equipment or products.

General requirements for the production of BLS

- In order to prevent undesirable changes in properties that may occur as a result of multiple passages or a large number of generations, the production of biological pharmaceutical substances and medicinal products obtained from microorganism cultures, cell cultures or propagation in embryos, tissues and organs of animals should be based on a system of master and working seed cultures and/or cell banks.
- The number of generations (doubles, passages) between the seed culture or cell bank and the biological pharmaceutical substance or medicinal product must comply with the requirements of the specifications in the registration dossier or clinical trial protocol. Seed cultures and cell banks should be established, stored and used in a manner that minimises the risk of contamination or alteration (e.g. stored in sealed containers in liquid nitrogen). The establishment of seed culture and cell bank systems, including master and working seed cultures, should be part of the life cycle management of the medicinal product and should be carried out under appropriate conditions.
- The cells and materials of biological origin used in the production process must be characterized and meet the requirements of microbiological and viral safety in accordance with the General Pharmacopoeia Monograph “Requirements for cell culture substrates for the production of biological medicinal products”.

Conditions for ensuring the quality of medicinal

- in production, only studied, genetically stable production strains of microbes are used, characterized and deposited in official collections, annually monitored for all biological properties, in accordance with regulated requirements; in this case, the genetic stability of the production strain is a criterion limiting the number of passages of the microbe

Conditions for ensuring the quality of medicinal

- use adequate nutrient media with high growth properties (raw materials, reagents and reactants used in the production of nutrient media must have certificates confirming their quality);
- use cell cultures in accordance with WHO recommendations, deposited in official collections and approved for use in production (when culturing cells, the use of native human blood serum, as well as antibiotics of the penicillin group, is not permitted)
- animals and birds, chicken embryos used for the production of biologically active medicinal products are obtained from farms that are free from bacterial, viral, prion and other diseases dangerous to humans, which is confirmed by veterinary certificates and certificates from a veterinary laboratory on the sanitary condition of the livestock, including microbiological and biochemical controls (OFS "Immunoglobulins and serums (antibodies) heterologous");

Conditions for ensuring the quality of medicinal

- - when producing medicinal products from human blood plasma and cells and organs, the requirements imposed on the donor's health status must be met (OFS "Medicinal products from human blood plasma");
- -BLPs that include donor tissues or cells must comply with the requirements of the legislation of the Russian Federation in terms of traceability, notification of the authorized federal executive body about adverse reactions and clinical cases during therapy, as well as in terms of technical requirements for the identification, processing, protection, storage and transportation of donor tissues and cells.

Ṣtages of biological drug research

- Preclinical. Both in vitro and in vivo testing are performed. The drug's activity, toxicity, minimum toxic doses, manifestations of toxicity over time, and receptor binding are assessed.
- Clinical. Conducted with the participation of large groups of people. The effectiveness of use, side and unwanted effects are assessed. From this point of view, the registration scheme for a biopreparation is similar to that of a drug obtained through chemical synthesis. Only after a comprehensive dossier has been compiled is the drug allowed for registration.