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# Microbiological landscape and parameters of antibiotic resistance of pathogens in patients of neonatal intensive care units

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Neonatal infections remain one of the significant causes of infant mortality in the world. The change in the spectrum of pathogens, as well as their sensitivity to the main antibacterial drugs (ABDs), is a dynamically occurring process, characterized by a gradual increase in the proportion of the most dangerous pathogens, in particular, those belonging to the ESKAPE pathogen group. The study of the structure of pathogens and the parameters of their antibiotic resistance is the main tool for increasing the effectiveness of antibiotic therapy.

**The aim**. To analyze the structure of pathogens of nosocomial infections in patients of neonatal intensive care units (NICU) and assess the parameters of their antibiotic resistance.

**Materials and methods**. A retrospective epidemiological study of data from May 1, 2022 to May 1, 2024 of the laboratory information system LIS-Alice of the Kommunarka Center (Moscow, Russia) and medical documentation of patients with identified growth of microorganisms (MOs) in bacteriological cultures was carried out.

**Results.** The total number of crops was 5179, MOs growth was noted in 39.3% (*n*=2036) obtained from 734 patients, of which 87.1% were premature. Gram-positive pathogens were found in 59.6%. The top 5 identified MOs were: *S. epidermidis* (*n*=386 — 19%), *S. haemolyticus* (*n*=264 — 13%), *S. aureus* (*n*=218 — 10.7%), *K. pneumoniae* (n=210 - 10.3%) and *E. coli* (*n*=188 — 9.2%). The proportion of MOs belonging to the ESKAPE group was 28.6% (*S. aureus* — 10.7%; *K. pneumoniae* — 10.3%; *Enterobacter spp.* — 3.6%; *P. aeruginosa* — 2.3%; *A. baumannii* — 1.1%; *E. faecium* — 0.5%). Among *Staphylococcus spp*. — 71.2% were resistant to oxacillin, 53.9% — to gentamicin. At the same time, 100% sensitivity to any of the tested ABDs was not detected. The highest rates of resistance to oxacillin were observed in *S. epidermidis* (93.8%) and *S. haemolyticus* (86.7%). Also, 17% of *S. aureus* strains were resistant to oxacillin. Among *K. pneumonia* 48.8% were resistant to ceftazidime and 100% to ampicillin; *E. coli* 28.2% of strains were resistant to ceftazidime, 64.9% to ampicillin, 28.2% to sulfamethoxazole trimethoprim.

**Conclusion.** We found a high frequency of pathogen isolation (with a predominance of gram-positive pathogens) in newborns hospitalized in the ICU (mean gestational age <35 weeks). The results demonstrate alarming trends in relation to MOs resistance parameters and indicate the need for dynamic monitoring of the sensitivity of pathogens to the main ABDs used in the ICU.

**Keywords:** pathogens of neonatal infection; antibiotic resistance; neonatal sepsis; ESKAPE group; *Staphylococcus spp.*, *Klebsiella spp.*, *Escherichia coli*

**Abbreviations:** ABDs — antibacterial drugs; BAL — bronchoalveolar lavage; CI — confidence interval; CoNS — coagulase-negative staphylococci; LIS — laboratory information system; MOs — microorganisms; NS — neonatal sepsis; ICU — intensive care unit; OR — odds ratio; EDA — endotracheal aspirate; MALDI-TOF - matrix-assisted laser desorption / ionization.

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# Микробиологический пейзаж и параметры антибиотикорезистентности возбудителей у пациентов отделений реанимации и интенсивной терапии новорожденных

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Неонатальные инфекции остаются одной из значимых причин младенческой смертности в мире. Изменение спектра возбудителей, а также их чувствительности к основным антибактериальным препаратам (АБП) является динамически протекающим процессом, характеризующимся постепенным ростом удельного веса наиболее опасных возбудителей, в частности, относящихся к группе ESKAPE-патогенов. Изучение структуры патогенов и параметров их антибиотикорезистентности является основным инструментом повышения эффективности антибиотикотерапии.

**Цель**. Проанализировать структуру возбудителей нозокомиальных инфекций пациентов отделений реанимации и интенсивной терапии (ОРИТ) новорождённых и оценка параметров их антибиотикорезистентности.

**Материалы и методы.** Проведено ретроспективное эпидемиологическое исследование данных за период с 1 мая 2022 по 1 мая 2024 гг. лабораторной информационной системы ЛИС-Алиса ГБУЗ «ММКЦ «Коммунарка» ДЗМ и медицинской документации пациентов с выявленным ростом микроорганизмов (МО) в бактериологических посевах.

**Результаты.** Общее число посевов составило 5179. Рост МО отмечен в 39,3% (*n*=2036) — были получены от 734 пациентов, из них 87,1% — недоношенные). Грамположительная микрофлора обнаружена в 59,6%. Топ-5 идентифицированных МО составили: *S. epidermidis* (*n*=386 — 19%), *S. haemolyticus* (*n*=264 — 13%), *S. aureus* (*n*=218 — 10,7%), *K. pneumoniae* (*n*=210 — 10,3%) и *E.coli* (*n*=188 — 9,2%). Доля МО, относящихся к группе ESKAPE, составила 28,6% (*S. aureus* — 10,7%; *K. pneumoniae* — 10,3%; *Enterobacter spp.* — 3,6%; *P. aeruginosa* — 2,3%; *A. baumannii* — 1,1%; *E. faecium* — 0,5%). Среди *Staphylococcus spp.* — 71,2% были резистенты к оксациллину, 53,9% — к гентамицину. При этом 100% чувствительности ни к одному из протестированных АБП выявлено не было. Максимально высокие показатели резистентности к оксациллину имели *S. epidermidis* (93,8%) и *S. haemolyticus* (86,7%). Также 17% штаммов *S. aureus* были резистентны к оксациллину. Среди *K. pneumonia* 48,8% были резистентны к цефтазидиму и 100% — к ампициллину; *E. coli* 28,2% штаммов были резистентны к цефтазидиму, 64,9% — к ампициллину, 28,2% — к сульфаметоксазолу / триметоприму.

**Заключение. Установлена высокая частота** выделения патогенов (с преобладанием грамположительной микрофлоры) у новорождённых, госпитализированных в ОРИТ (средний гестационный возраст <35 нед.). Результаты демонстрируют тревожные тенденции в отношении параметров резистентности МО и свидетельствуют о необходимости динамического мониторинга чувствительности возбудителей к основным АБП, применяющимся в ОРИТ.

**Ключевые слова:** возбудители инфекции новорождённых; антибиотикорезистентность; неонатальный сепсис; группа ESKAPE; стафилококки; клебсиеллы; кишечная палочка

**Список сокращений:** АБП — антибактериальные препараты; БАЛ — бронхоальвеолярный лаваж; ДИ — доверительный интервал; КНС — коагулазонегативные стафилококки; ЛИС — лабораторно-информационная система; МО — микроорганизмы; НС — неонатальный сепсис; ОРИТ — отделение реанимации и интенсивной терапии; ОШ — отношение шансов; ЭДТА — эндотрахеальный аспират; MALDI-TOF — матрично-активированная лазерная десорбция / ионизация.

## INTRODUCTION

Neonates are a special category of patients whose physiological characteristics and pathological processes determine high risks of infection with various pathogens, primarily bacterial ones. In turn, neonatal infections have a significant negative prognosis both in the short term (prolonged hospitalization, development of sepsis, death) [1–4] and in the long term perspective. Published data reveal a high risk of damage to the central nervous system in newborns with severe

infections in the first days after birth, which leads to disruptions in further development [5]. Studies have demonstrated a decrease in cognitive and motor functions, as well as hearing in children who have suffered neonatal infections [6]. It was established that neonatal sepsis (NS) is a risk factor for the development of severe functional disabling disorders in children aged 24 months (odds ratio, OR=3.68, 95% confidence interval, CI: from 1.2 to 11.2, *p*=0.021) [7]. NS occurs significantly more often in the presence of infection in the mother, during invasive procedures, and premature rupture of membranes [8–10]. The newborn characteristics as primarily gestational age and weight deserve special attention. A meta-analysis of 15 studies determined that OR for the development of NS at a gestational age of less than 37 weeks is 2.05 (95% CI: from 1.40 to 2.99), and with premature rupture of membranes — 11.14 (95% CI: from 5.54 to 22.38) [11]. Another meta-analysis identified a significant links between the development of NS and low birth weight (OR=1.42 (95% CI: from 1.07 to 1.88)) [12]. In a retrospective analysis of medical records of newborns, low birth weight and low gestational age were identified as independent risk factors for severe nosocomial infections [13].

In terms of assessing the role of pathogens, it is necessary to note the role of colonization of pregnant women with various microorganisms (MOs). Thus, in the work of Olenev et al. (2022), the widespread colonization of group B streptococcus and its significant negative consequences for newborns were demonstrated [14]. Published data established a link between infection with certain pathogens and an increased risk of developing NS [15]. Bacterial pathogens associated with early NS include primarily group B streptococci, *Escherichia coli*, *Listeria monocytogenes*, *Klebsiella* spp., *Pseudomonas* spp. and *Haemophilus influenzae* [15–17]. An association with the development of late NS has been demonstrated for coagulase-negative staphylococci (CNS), Staphylococcus aureus, the above-mentioned gram-negative pathogens, and Enterobacter spp [15, 18].

Microbiological monitoring of newborns at risk for infection (preterm low birth weight infants) is an important tool for reducing infant mortality [19]. Published data indicate that approximately 67,000 newborns die worldwide every day, with neonatal infections playing a significant role [20]. According to data collected within the framework of the CHAMPS — a global program for monitoring the health of children in regions with the highest infant mortality[1] — the most common cause of death in newborns were infections (40%). The second place was determined for prematurity (32%) and the third for respiratory distress syndrome (28%) [21]. Timely detected pathogens and the study of their antibiotic resistance parameters are an important condition for the effectiveness of antibiotic therapy and, accordingly, a positive clinical outcome.

**THE AIM.** To analyze the structure of pathogens of nosocomial infections in patients of neonatal intensive care units and to assess the parameters of their antibiotic resistance.

## MATERIALS AND METHODS

### Study design

A retrospective epidemiological study was conducted using data from the LIS-Alice laboratory information system (search keywords: “NICU-1”, “NICU-2”; total number of identified cultures — 5179) of the State Budgetary Healthcare Institution “MMCC “Kommunarka” of the Moscow Health Department, as well as medical documentation of patients who had a detected growth of MOs in bacteriological cultures (*n*=734). The study period was from May 1, 2022 to May 1, 2024.

NICU-1 is a neonatal intensive care unit of the first stage of nursing. Admission criteria: newborns with very low (<2.5 kg) and extremely low body weight (<1 kg), low gestational age, in critical condition in the neonatal period, especially with acute respiratory failure.

NICU-2 is a neonatal intensive care unit of the second stage of nursing. Admission criteria: patients after stabilization of their condition in NICU-1 (requiring a stay in the ICU for more than 7 days), admitted for further nursing, as well as patients transferred from the neonatal pathology unit due to deterioration of their condition (development of respiratory failure, heart failure requiring vasopressor support, condition after surgery).

### Research methodology

LIS-Alice (locus of material collection, presence of growth, pathogen, data on its sensitivity / resistance to ABP) was used as a source of data on patient cultures and identified parameters of antibiotic resistance. Patient medical histories (gender, age, body weight, Apgar score at birth) were used as a source of demographic, anthropometric and clinical data. The collecting of samples was carried out by physicians as part of standard patient management (upon admission to the

[1] Child Health and Mortality Prevention Surveillance. – [Электронный ресурс]. – Режим доступа: https://champshealth.org/

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NICU for the purpose of microbiological monitoring, as well as in case of signs of infection). For the identification of MOs, the MALDI-TOF mass spectrometry (matrix-assisted laser desorption/ ionization) method was used in the microbiology laboratory. Sensitivity to antibacterial drugs was determined by the automated disk diffusion method.

### Ethics approval

The study approval from Local Ethics Committees of the Medical Institute of the Peoples' Friendship University of, extract from Protocol No. 24 dated January 18, 2024; Moscow Multidisciplinary Clinical Center "Kommunarka, extract from Protocol No. 2 dated 13.02.2024).

### Statistical processing

Statistical data processing was performed using Microsoft Excel 2019 software. Descriptive statistics were used for all analyzed indicators (mean and standard deviation (SD), minimum (min) and maximum values (max), median (Me), interquartile range (IQR) were determined). Qualitative variables were described using absolute (*n*) and relative (%) values.

## RESULTS

The total number of cultures identified during the study period (two years) was 5179. Of these, microbial growth was observed in 39.3% (*n*=2036). One MO was detected in 74.3% of cultures (*n*=545); 2 or more MOs — in 25.7% (*n*=189).

The total number of patients from whom cultures with growth were obtained was 734. Of these, 87.1% were premature infants (*n*=639). The average Apgar score at 1 minute was 4.9±2.2 (min=1, max=8, Me=5, IQR=1.9); at 5 min 6.0±1.6 (min=3, max=9, Me=6). Female gender was in 66.6% (*n*=489), male in 33.4% (*n*=245).

The mean gestational age of the total population of neonates according to medical records was 34.6±4.8 weeks (min=20.4 weeks, max=54.3 weeks, Me=35.1 weeks, IQR=8.8). The mean birth weight was 2970.7±1271.9 g (min=360, max=4125, Me=3705, IQR=1746.5). Extremely low birth weight (<1000 g) was observed in 14.2% (*n*=104), very low (1000–1499 g) —in 26.7% (*n*=196), low (1500–2499 g) — in 21.1% (*n*=155), normal (>2500 g) — in 38% (*n*=279).

Patient characteristics depending on hospitalization in NICU-1 and NICU-2 are presented in Table 1.

The mean duration of hospitalization at the time of detection of MO was 12.6±12.4 (min=0, max=97.3, Me=6, IQR=17.1) days.

In the overall structure of cultures (both with and without growth), the analysis of the loci of biomaterial collection revealed the dominance of samples from the pharynx (*n*=2669, 51.5%, of which growth-positive were 1434, 53.7%). Next came blood (*n*=1431, 27.6%, of which growth-positive were 170, 11.8%) and endotracheal aspirate (*n*=451, 8.7%, of which growth-positive were 180, 39.9%). Among the cultures with detected growth, the pharyngeal swab was leader (*n*=1334, 65.5%), the second place was determined for the rectal swab (*n*=212, 10.4%), and the third for the endotracheal aspirate swab (*n*=181, 8.9%). The contribution of each locus to the total structure of cultures is presented in Table 2.

The next stage of the analysis was devoted to assessing the proportion of gram-positive and gram-negative MOs in the overall structure. Gram-positive microflora was detected in 59.6% (*n*=1213), gram-negative — in 40.4% (*n*=823). The proportion of detected gram-positive and gram-negative MOs depending on the locus of biomaterial collection is presented in Figure 1.

We found a predominance of gram-positive MOs in the blood (*n*=143, 89.4%) and pharyngeal swab (*n*=821, 61.5%), while gram-negative microorganisms dominated in endotracheal aspirate (EDTA; *n*=105, 58.0%) and rectal swab (*n*=118, 55.7%)

In the overall structure of MOs, the absolute predominance was detected for *Staphylococcus* spp. (46.2%), and second were isolates of *Klebsiella* spp. (15.32%). The main groups of microorganisms identified as a result of the analysis are presented in Figure 2.

Studying the spectrum of MOs isolated from various loci, it was found that for pharyngeal swabs and blood samples, the first place among all pathogens was occupied by strains of *S. epidermidis*, while in the rectal swab and EDTA the leader was *K. pneumoniae*. It is noteworthy that in EDTA the second place in terms of detection frequency belonged to a rather rare pathogen, *Stenotrophomonas maltophilia*, while the first place was shared by *K. pneumoniae* and *S. epidermidis* (13.8% for each), a detailed picture is presented in Figure 3.

The analysis of the isolated pathogens revealed a large proportion of representatives of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.). The proportion of cultures with ESKAPE pathogens was 28.6% (*n*=582). The structure of the ESKAPE MOs identified in our study is presented in Figure 4.

Determination of the frequency of occurrence

of each pathogen in the overall structure of cultures allowed us to determine the top five pathogens identified in neonates in the NICU. They included *S. epidermidis* (*n*=386; 19%), *S. haemolyticus* (*n*=264; 13%), *S. aureus* (*n*=218; 10.7%), *K. pneumoniae* (*n*=210; 10.3%) and *E. coli* (*n*=188; 9.2%).

The detailed structure of pathogens identified in samples from different loci is presented below. Staphylococci were the leading pathogens in pharyngeal swabs (48.9%). Information on the proportion of other pathogens identified from this locus is presented in Table 3.

In the rectal swabs, gram-negative microflora dominated. However, assessing the contribution of each MO, we registered that the first place belonged to staphylococci (31.6%), and *Klebsiella* spp. occupied the second place (29.2%). The overall structure of pathogens detected in rectal swabs is presented in Table 4.

In the general structure of pathogens detected in EDTA swabs, *Staphylococcus* spp. (26.0%) and *Klebsiella* spp. (20.4%) also led. The general structure of pathogens detected in EDTA is presented in Table 5.

The blood samples showed a predominance of gram-positive MOs, with the proportion of staphylococci in the overall structure being 73.8%. Detailed data on the structure of pathogens detected in the blood are presented in Table 6.

The next stage of our work was devoted to assessing the sensitivity of the identified MOs to ABDs. Since the main gram-positive MOs were staphylococci, enterococci, streptococci and corynebacteria, below we present the results of a combined analysis of sensitivity to ABDs for these groups of pathogens (Table 7). The most significant results included the detection of staphylococcal resistance: 71.2% of all strains were resistant to oxacillin and more than half of the strains were resistant to gentamicin (53.9%).

The results of the analysis of the sensitivity of the main gram-negative pathogens to different ABDs are presented in Table 8. They demonstrated a higher conditional average level of antibiotic resistance compared to gram-positive pathogens. 88.9% of *Klebsiella* spp. strains were resistant to ampicillin, more than a third — to the third-generation cephalosporin ceftazidime (34.7%). Mentioned ABDs are also of interest in terms of E. coli resistance: 64.9% of strains were resistant to the first, 28.2% to the second. For *Acinetobacter* spp. and *Enterobacter* spp., we did not find significant changes in sensitivity to ABDs. For *P. aeruginosa*, the analysis established trends towards increasing resistance to all tested ABDs.

The top MOs in the structure were *S. epidermidis* and *S. haemoliticus*. The study of their ABDs sensitivity revealed a significant proportion of oxacillin-resistant strains (93.8 and 86.7%, respectively) and gentamicin-resistant strains (70.2 and 80.2%, respectively), detailed data are presented in Table 9.

Among the identified pathogens, almost a third belonged to the ESKAPE group. These MOs usually cause severe infectious processes, and their presence is usually accompanied by a high level of antibiotic resistance. In this regard, we conducted a separate analysis of the sensitivity parameters of *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp.; the results are presented in Table 10 (for gram-positives) and Table 11 (for gram-negatives).

Considering that the pathogens to the ESKAPE group were determined in different loci, which implies different localization of the infectious process and may also indicate the degree of its generalization, we then conducted a comparative analysis of the antibiotic resistance of individual MOs of this group depending on the source of biomaterial collection (*A. baumannii* was not included in the analysis, since no significant changes in sensitivity in the overall structure were found for it). The results revealed the highest resistance rates of gram-positive MOs in endotracheal aspirate samples. *K. pneumoniae* strains showed a fairly high resistance to ceftazidime in all samples, and 100% resistance to ampicillin. The most resistant *P. aeruginosa* strains were isolated primarily from samples obtained from rectal swabs. Detailed information is presented in Figures 5–8.

## DISCUSSION

Our results revealed that population of patients hospitalized in the NICU was represented mainly by premature neonates (87.1%) with low birth weight (birth weight ≤ 2499 g was detected in 62%). Accordingly, most patients were at risk of infection with bacterial pathogens. In general, published data indicate a fairly high incidence of nosocomial infections in premature infants (gestational age<32 weeks, birth weight <1500 g): from 5.6% to 34.4% during the first 120 days of life [22]. The localization and type of infections are variable, but the most common in the global practice of neonatal care are bloodstream infections (frequency from 5.6 per 1000 days of central venous catheter use to 7.3 per 1000 days of umbilical catheter use) [23], and ventilator-associated pneumonia (frequency 7.8 per 1000 days of artificial ventilation use) [24].

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**Table 1 – Characteristics of patient with growth-positive cultures**

| Parameter | NICU-1 (*n*=311) | NICU-2 (*n*=423) |
|---|---|---|
| Age at the time of hospitalization, weeks | | |
| M + SD | 33.8±4.5* | 36.7±5.1** |
| min | 20.4 | 27.0 |
| max | 41.6 | 54.3 |
| Me (IQR) | 35.0 (9.3) | 35.5 (7.9) |
| Body weight at the time of hospitalization, g | | |
| M+SD | 2185.2±1022.8 | 2269.0±926.8 |
| min | 360.0 | 661.0 |
| max | 3940.0 | 4125.0 |
| Me (IQR) | 2074.5 (1943.5) | 2117.5 (1982.5) |

Note: * gestational age; ** postconceptual age. NICU — neonatal intensive care unit.

**Table 2 – Contribution of each locus to the structure of cultures**

| Locus | Culture-negative + culture-positive | | Culture-positive | |
|---|---|---|---|---|
| | *n* (total 5179) | % | *n* (total 2036) | % |
| Pharynx | 2669 | 51.5 | 1334 | 65.5 |
| Rectum | 362 | 7.0 | 212 | 10.4 |
| Endotracheal aspirate | 451 | 8.8 | 181 | 8.9 |
| Blood | 1431 | 27.6 | 160 | 7.9 |
| Urine | 137 | 2.6 | 97 | 4.8 |
| Vascular catheter | 94 | 1.8 | 24 | 1.2 |
| Skin | 25 | 0.5 | 20 | 1.0 |
| Other (CSF, BAL, gastric contents, conjunctival secretions) | 10 | 0.2 | 8 | 0.4 |

Note: BAL — bronchoalveolar lavage.

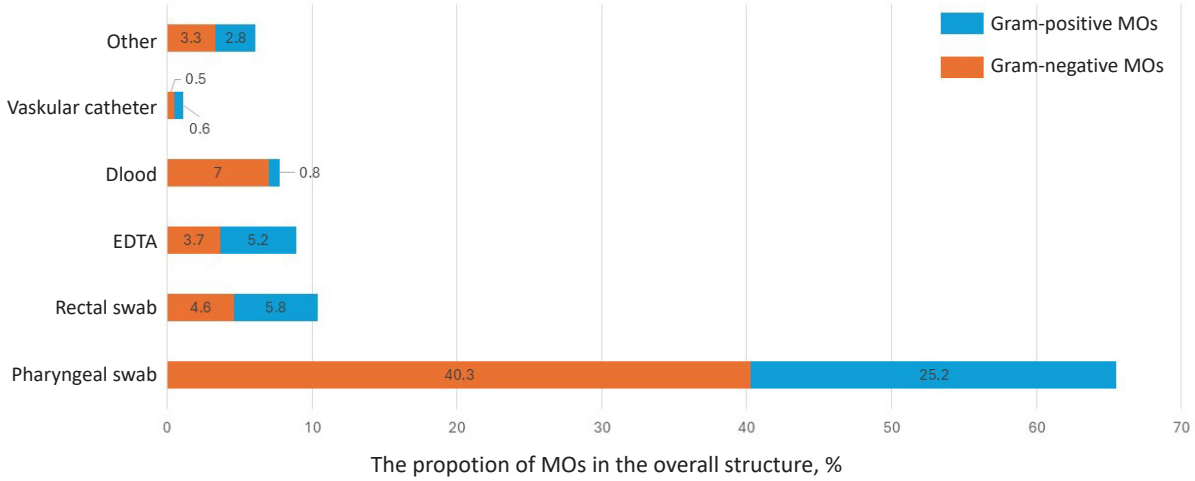


**Figure 1 – The proportion of detected gram-positive and gram-negative Mos depending on the locus of biomaterial collection.**

Note: EDTA — endotracheal aspirate; MOs — microorganisms.

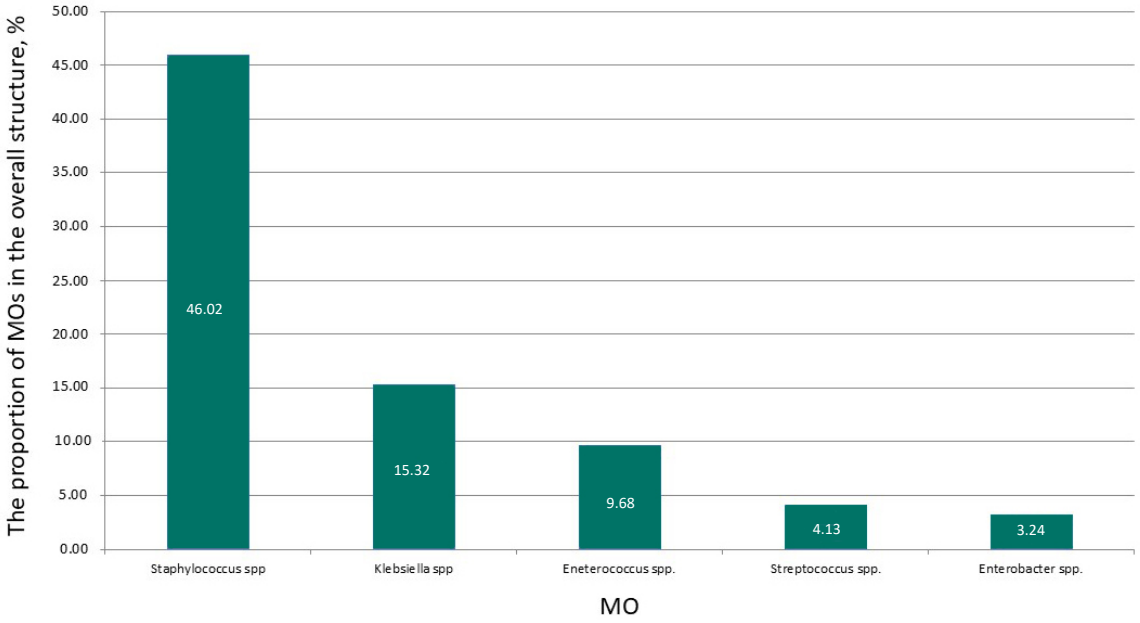


**Figure 2 – The main groups of MOs identified in the analysis of neonatal cultures in the neonatal intensive care units.**

Note: MOs — microorganisms.

**Table 3 – Structure of pathogens identified in throat swabs**

| Pharyngeal swab | *n* (total 1334) | % |
|---|---|---|
| Gram-positive MOs | 821 | 61.5 |
| *Staphylococcus* spp.: | 652 | 48.9 |
| • *S. epidermidis* | 250 | 18.7 |
| • *S. haemoliticus* | 205 | 15.4 |
| • *S. aureus* | 162 | 12.1 |
| • *S. hominis* | 26 | 1.9 |
| • *S. lugdunensis* | 8 | 0.6 |
| • *S. capitis* | 1 | 0.1 |
| *Enterococcus* spp.: | 102 | 7.6 |
| • *E. faecalis* | 97 | 7.3 |
| • *E. faecium* | 5 | 0.4 |
| *Streptococcus* spp.: | 54 | 4.0 |
| • *S. agalactiae* | 49 | 3.7 |
| • *S. anginosus* | 8 | 0.6 |
| *Corynebacterium* spp. | 10 | 0.7 |
| *Listeria monocytogenes* | 3 | 0.2 |
| Gram-negative MOs | 513 | 38.5 |
| *Klebsiella* spp.: | 189 | 14.2 |
| • *K.pneumoniae* | 128 | 9.6 |
| • *K. oxytoca* | 42 | 3.1 |
| • *K. variicola* | 1 | 0.1 |
| • *K. aerogenes* | 18 | 1.3 |
| *E. coli* | 167 | 12.5 |
| *Enterobacter* spp. | 49 | 3.7 |
| *Acinetabacter* spp.: | 33 | 2.5 |
| • *A. baumannii* | 18 | 1.3 |
| • *A. albensis* | 1 | 0.1 |
| • *A. pittii* | 6 | 0.4 |
| *Stenotrophomonas maltophilia* | 24 | 1.8 |
| *P. aeruginosa* | 20 | 1.5 |
| *Citrobacter* spp. | 20 | 1.5 |
| *H. influenzae* | 7 | 0.5 |
| *P. mirabilis* | 3 | 0.2 |
| *Morganella morganii* | 1 | 0,1 |

Note: MOs — microorganisms.

**Table 4 – Overall structure of pathogens detected in rectal swabs**

| Rectal swab | *n* (total 212) | % |
|---|---|---|
| Gram-positive MOs | 94 | 44.3 |
| *Staphylococcus* spp.: | 67 | 31.6 |
| • *S. epidermidis* | 24 | 11.3 |
| • *S. aureus* | 22 | 10.4 |
| • *S. haemoliticus* | 20 | 9.4 |
| • *S. warneri* | 1 | 0.5 |
| *Enterococcus* spp.: | 27 | 12.7 |
| • *E. faecalis* | 19 | 9.0 |
| • *E. faecium* | 8 | 3.8 |
| Gram-negative MOs | 118 | 55.7 |
| *Klebsiella* spp.: | 62 | 29.2 |
| • *K. Pneumoniae* | 44 | 20.8 |
| • *K. Oxytoca* | 11 | 5.2 |
| • *K. Aerogenes* | 7 | 3.3 |
| *P. aeruginosa* | 17 | 8.0 |
| *E. coli* | 12 | 5.7 |
| *Acinetobacter* spp.: | 4 | 1.9 |
| • *A. lwoffii* | 1 | 0.5 |
| • *A. baumannii* | 1 | 0.5 |
| *Enterobacter* spp. | 14 | 6.6 |
| *Stenotrophomonas maltophilia* | 4 | 1.9 |
| *Serratia* spp. | 3 | 1.4 |
| *Proteus* spp. | 2 | 0.9 |

Note: MOs — microorganisms.

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**Table 5 – General structure of pathogens detected in endotracheal aspirate**

| EDTA | *n* (total 181) | % |
|---|---|---|
| Gram-positive MOs | 76 | 42.0 |
| *Staphylococcus* spp.: | 47 | 26.0 |
| • *S. epidermidis* | 25 | 13.8 |
| • *S. aureus* | 14 | 7.7 |
| • *S. haemoliticus* | 8 | 4.4 |
| *Streptococcus* spp.: | 14 | 7.7 |
| • *S. agalactiae* | 10 | 5.5 |
| • *S. anginosus* | 1 | 0.6 |
| • *S. gordonii* | 1 | 0.6 |
| • *S. salivarius* | 1 | 0.6 |
| • *S. lutetiensis* | 1 | 0.6 |
| *Enterococcus* spp.: | 8 | 4.4 |
| • *E. faecalis* | 7 | 3.9 |
| • *E. faecium* | 1 | 0.6 |
| *Corynebacterium* spp. | 4 | 2.2 |
| *Listeria monocytogenes* | 3 | 1.7 |
| Gram-negative MOs | 105 | 58.0 |
| *Klebsiella* spp.: | 37 | 20.4 |
| • *K. pneumoniae* | 25 | 13.8 |
| • *K. aerogenes* | 10 | 5.5 |
| • *K. oxytoca* | 7 | 3.9 |
| *Stenotrophomonas maltophilia* | 18 | 9.9 |
| *P. aeruginosa* | 14 | 7.7 |
| *Enterobacter* spp.: | 10 | 5.5 |
| *E. coli* | 8 | 4.4 |
| *Serratia* spp. | 6 | 3.3 |
| *Acinetobacter* spp. | 5 | 2.8 |
| • *A. baumannii* | 3 | 1.7 |
| • *A. junii* | 2 | 1.1 |
| *Citrobacter* spp. | 3 | 1.7 |
| *H. influenzae* | 1 | 0.6 |
| *Ralstonia pickettii* | 1 | 0.6 |
| *Proteus mirabilis* | 1 | 0.6 |
| *Delftia acidovorans* | 1 | 0.6 |

Note: EDTA — endotracheal aspirate; MOs — microorganisms.

**Table 6 – Overall structure of pathogens detected in the blood samples**

| Blood | *n* (total 160) | % |
|---|---|---|
| Gram-positive MOs | 143 | 89.4 |
| *Staphylococcus* spp.: | 118 | 73.8 |
| • *S. epidermidis* | 71 | 44.4 |
| • *S. haemoliticus* | 31 | 19.4 |
| • *S. aureus* | 15 | 9.4 |
| • *S. capitis* | 1 | 0.6 |
| *Streptococcus* spp.: | 14 | 8.8 |
| • *S. agalactiae* | 9 | 5.6 |
| • *S. anginosus* | 1 | 0.6 |
| • *S. gordonii* | 1 | 0.6 |
| • *S. salivarius* | 1 | 0.6 |
| • *S. lutetiensis* | 1 | 0.6 |
| • *S. pneumoniae* | 1 | 0.6 |
| *Corynebacterium* spp. | 4 | 2.5 |
| *Enterococcus* spp.: | 4 | 2.5 |
| • *E. faecalis* | 4 | 2.5 |
| • *E. faecium* | 0 | 0.0 |
| *Listeria monocytogenes* | 3 | 1.9 |
| Gram-negative MOs | 17 | 10.6 |
| *Klebsiella* spp.: | 8 | 5.0 |
| • *K. pneumoniae* | 8 | 5.0 |
| *P. aeruginosa* | 2 | 1.3 |
| *Enterobacter* spp.: | 2 | 1.3 |
| *E. coli* | 1 | 0.6 |
| *H. influenzae* | 1 | 0.6 |
| *Acinetobacter* spp.: | 1 | 0.6 |
| • *A. baumannii* | 1 | 0.6 |
| • *A. junii* | 0 | 0.6 |
| • *A. lwoffii* | 1 | 0.6 |
| • *Citrobacter* spp. | 1 | 0.6 |

Note: MOs — microorganisms.

**Table 7 – Parameters of antibacterial drugs sensitivity of the main gram-positive microorganisms**

| ABD | Proportion (%) R/S, MOs | *Staphylococcus* spp. (*n*=894) | *Enterococcus* spp. (*n*=141) | *Streptococcus* spp. (*n*=86) | *Corynebacterium* spp. (*n*=18) |
|---|---|---|---|---|---|
| Oxacillin | R | 71.2 | NA | 0 | NA |
| | S | 28.9 | NA | 100 | NA |
| Tigecycline | R | 0.9 | 0 | 0 | NA |
| | S | 99.1 | 100 | 100 | NA |
| Vancomycin | R | 1.8 | 0 | 0 | 0 |
| | S | 98.2 | 100 | 100 | 100 |
| Linezolid | R | 0.8 | 0 | 0 | 0 |
| | S | 99.2 | 100 | 100 | 100 |
| Gentamicin | R | 53.9 | NA | NA | NA |
| | S | 46.1 | NA | NA | NA |
| Ampicillin | R | NA | 7.1 | 0 | NA |
| | S | NA | 92.9 | 100 | NA |

Note: ABDs — antibacterial drugs; MOs — microorganisms; NA — not applicable; R — resistant; S — sensitive.

**Table 8 – Parameters of antibacterial drugs sensitivity of the main gram-negative microorganisms**

| ABDs | Proportion (%) R/I/S, MOs | *Klebsiella* spp. (*n*=296) | *E. coli* (*n*=188) | *Acinetobacter* spp. (*n*=57) | *Enterobacter* spp. (*n*=75) | *P. aeruginosa* (*n*=54) |
|---|---|---|---|---|---|---|
| Amikacin | R | 5.1 | 4.8 | 0 | 2.7 | 19.5 |
| | S | 95.9 | 95.2 | 100 | 97.3 | 80.5 |
| Cefepime | R | 7.6 | 3.2 | 10.5 | 2.7 | 30.5 |
| | I | 11.1 | 0.5 | 0 | 1.3 | 82.0 |
| | S | 81.3 | 96.3 | 89.5 | 96 | 14.8 |
| Meropenem | R | 6.2 | 0 | 3.5 | 0 | 20.1 |
| | S | 93.8 | 100 | 96.5 | 100 | 53.2 |
| Ceftazidime | R | 34.7 | 28.2 | NA | 16 | 30.5 |
| | I | 12.6 | 5.3 | NA | 1.3 | 65.8 |
| | S | 52.7 | 66.5 | NA | 82.7 | 3.7 |
| Gentamicin | R | 14.3 | 4.8 | 5,3 | 4 | NA |
| | S | 85.7 | 95.2 | 94,7 | 96 | NA |
| Ampicillin | R | 88.9 | 64.9 | NA | 100 | NA |
| | S | 11.1 | 35.1 | NA | 0 | NA |
| Sulfamethoxazole trimethoprim | R | 3.7 | 28.2 | 7.0 | 0 | NA |
| | S | 96.3 | 71.8 | 93.0 | 100 | NA |

Note: ABDs — antibacterial drugs; MOs — microorganisms; NA — not applicable; R — resistant; I — intermediate; S — sensitive.

**Table 9 – Parameters of antibacterial drugs sensitivity of *S. epidermidis* and *S. haemoliticus***

| ABDs | Proportion (%) R/S, MOs | *S. epidermidis* (*n*=386) | *S. haemoliticus* (*n*=264) |
|---|---|---|---|
| Oxacillin | R | 93.8 | 86.7 |
| | S | 6.2 | 13.3 |
| Tigecycline | R | 0.8 | 0.8 |
| | S | 99.2 | 99.2 |
| Vancomycin | R | 0 | 1.5 |
| | S | 100 | 98.5 |
| Linezolid | R | 0 | 3.4 |
| | S | 100 | 96.6 |
| Gentamicin | R | 70.2 | 80.2 |
| | S | 29.8 | 19.8 |

Note: ABDs — antibacterial drugs; MOs — microorganisms; R — resistant; S — sensitive.

**Table 10 – Parameters of antibacterial drugs sensitivity of gram-positive microorganisms of the ESKAPE group**

| ABDs | Proportion (%) R/S, MOs | *S. aureus* (*n*=218) | *E. faecium* (*n*=10) |
|---|---|---|---|
| Oxacillin | R | 17.1 | NA |
| | S | 82.9 | NA |
| Tigecycline | R | 0 | 0 |
| | S | 100.0 | 100 |
| Vancomycin | R | 4.5 | 0 |
| | S | 95.5 | 100 |
| Linezolid | R | 4.2 | 0 |
| | S | 95.8 | 100 |
| Gentamicin | R | 9.2 | NA |
| | S | 90.8 | NA |
| Ampicillin | R | NA | 72.2 |
| | S | NA | 27.8 |

Note: ABDs — antibacterial drugs; MOs — microorganisms; NA — not applicable; R — resistant; S — sensitive.

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**Table 11 – Parameters of antibacterial drugs sensitivity of gram-negative microorganisms of the ESKAPE group**

| ABDs | Proportion (%) R/I/S, MOs | *K. pneumoniae* (*n*=210) | *A. baumanni* (*n*=23) | *Enterobacter* spp. (*n*=75) | *P. aeruginosa* (*n*=54) |
|---|---|---|---|---|---|
| Amikacin | R | 2.0 | 0.0 | 2.7 | 19.5 |
| | S | 98.0 | 100.0 | 97.3 | 80.5 |
| Cefepime | R | 7.6 | 17.0 | 2.7 | 22.4 |
| | I | 0.0 | 0 | 1.3 | 64.1 |
| | S | 92.4 | 83.1 | 96 | 13.5 |
| Meropenem | R | 0.6 | 0.0 | 0 | 20.1 |
| | S | 99.4 | 100.0 | 100 | 53.2 |
| Ceftazidime | R | 48.8 | NA | 16 | 30.5 |
| | I | 7.0 | NA | 1.3 | 65.8 |
| | S | 44.2 | NA | 82.7 | 3.7 |
| Gentamicin | R | 6.9 | 0.0 | 4 | NA |
| | S | 93.1 | 100.0 | 96 | NA |
| Ampicillin | R | 100.0 | NA | 100 | NA |
| | S | 0.0 | NA | 0 | NA |
| Sulfamethoxazole trimethoprim | R | 3.7 | 6.2 | 0 | NA |
| | S | 96.3 | 93.8 | 100 | NA |

Note: ABDs — antibacterial drugs; MOs — microorganisms; NA — not applicable; R — resistant; I — intermediate; S — sensitive.

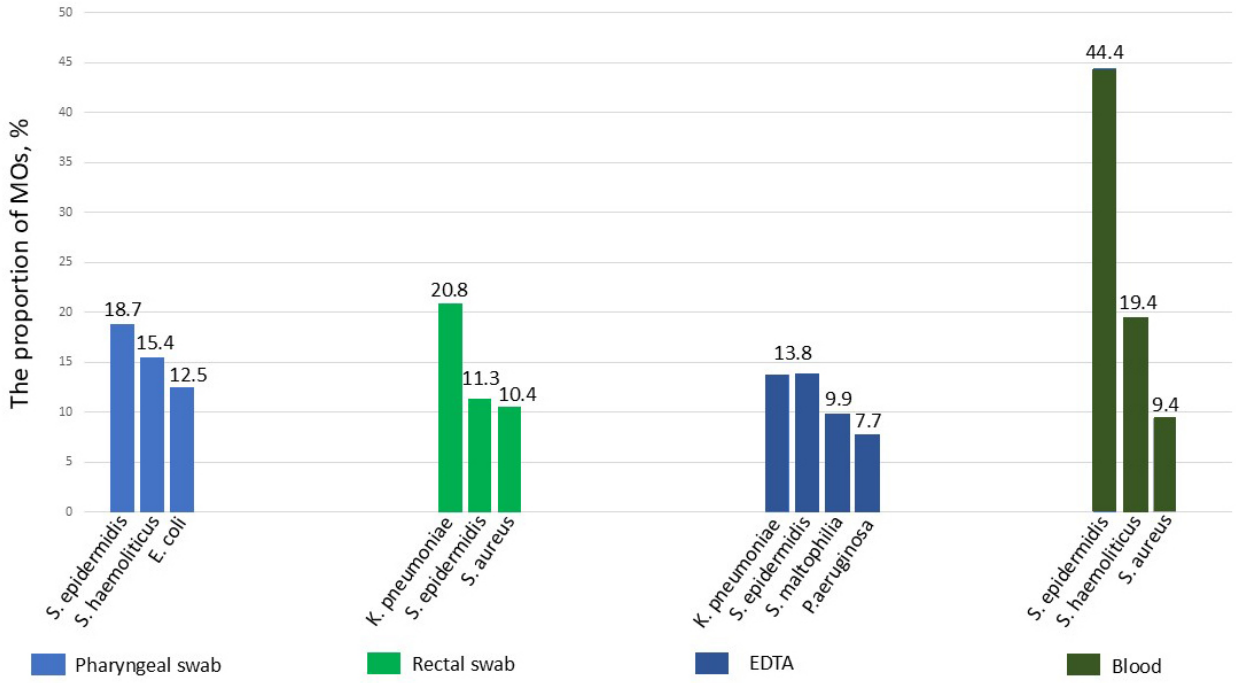


**Figure 3 – Leading microorganisms identified in the main loci of biomaterials collection.**
Note: MOs — microorganisms.

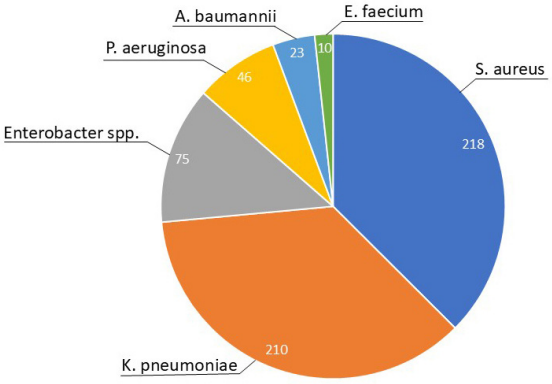


**Figure 4 – The structure of ESKAPE group pathogens.**
Note: The data is presented as absolute values.

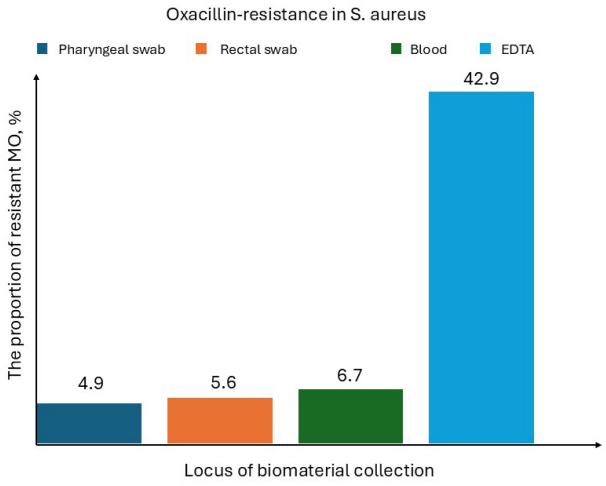


**Figure 5 – Oxacillin-resistance in *S. aureus* depending on the locus of biomaterial collection.**
Note: EDTA — endotracheal aspirate; MO — microorganism.

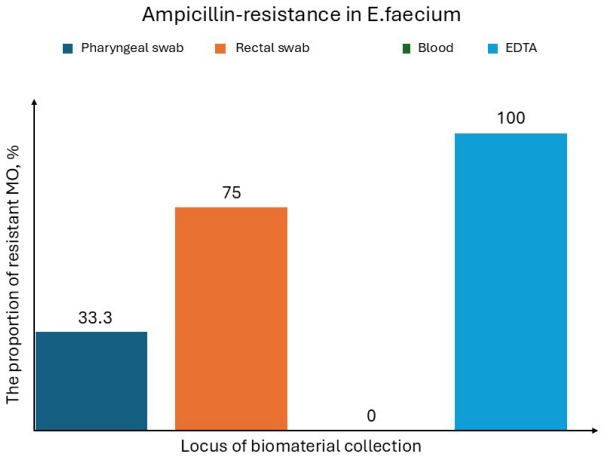


**Figure 6 – Ampicillin-resistance in *E. faecium* depending on the locus of biomaterial collection.**
Note: EDTA — endotracheal aspirate; MO — microorganism.

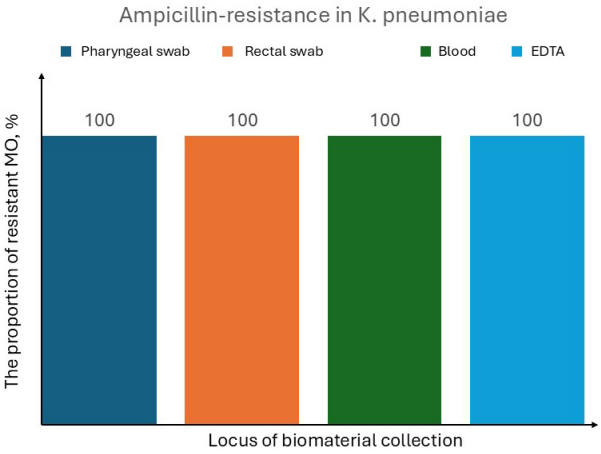


**Figure 7 – Ampicillin-resistance in *K. pneumoniae* depending on the locus of biomaterial collection.**
Note: EDTA — endotracheal aspirate; MO — microorganism.

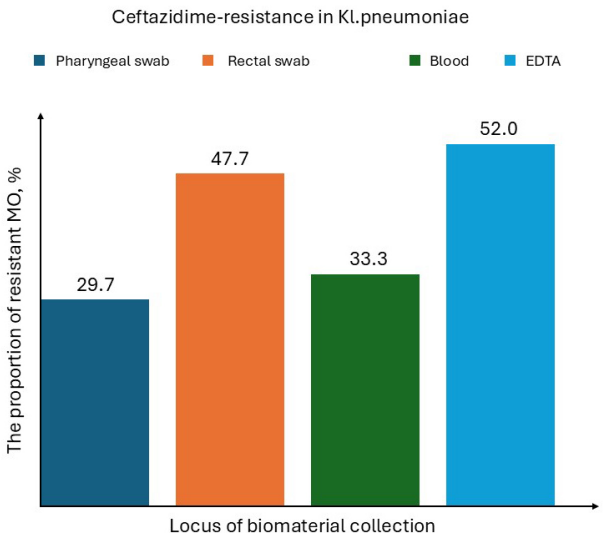


**Figure 8 – Ceftazidime-resistance in *K. pneumoniae* depending on the locus of biomaterial collection.**
Note: EDTA — endotracheal aspirate; MO — microorganism.

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The localization and nature of the infectious process depend on the locus of MO identification. Our study found a predominant growth of MO in samples from non-sterile loci (pharyngeal swab, rectal swab). More attention should be paid to the fact that growth was also recorded in loci that are normally sterile: in EDTA and blood, the latter indicating the development of bacteremia and possible sepsis. According to an analysis of 451,443 newborns in the United States, the frequency of nosocomial bacteremia was 2%, unadjusted incidence rate was 1.1 per 1000 patient-days (95% CI: 1.0 to 1.2), and a significantly higher incidence for neonates weighing less than 750 g was demonstrated (14.2 per 1000 patient-days (95% CI: 12.6 to 16.1) [25].

In the structure of the identified MOs, representatives of gram-positive microflora were in the lead – 59.6% (*n*=1213). In general, this is consistent with the results of previously published studies. According to a retrospective epidemiological study lasting 11 years (Sweden, 2006–2016), gram-positive MOs were identified four times more often in newborns with bloodstream infections compared to gram-negative MOs; the most common pathogens were represented by CNS (53.8%) [26]. In a prospective cross-sectional study that included newborns with NS, gram-positive MOs were identified in 53.4% [27]. According to a retrospective analysis of the spectrum of pathogens identified in newborns with NS (China), the majority also belonged to gram-positives, while in the overall structure the authors determined the leadership of CNS (41%) [28]. Similar results were obtained in a literature review (30 sources in the final analysis): CNS as NS pathogens were identified in 40.23% [29]. The results of a study of NS pathogens, which included a 20-year period (South Korea), are indicative. They established an absolute predominance of gram-positive MOs (75.3%), among which the main contribution was also made by CNS [30]. The top 5 pathogens identified in our study included *S. epidermidis* (*n*=386, 19.0%), *S. haemolyticus* (*n*=263; 12.9%), *S. aureus* (*n*=218; 10.7%), *K. pneumoniae* (*n*=210; 10.3%) and *E. coli* (*n*=188; 9.2%). *S. epidermidis*, a representative of the CNS, is characterized by a high frequency of detection in newborns in general (being a commensal, it colonizes the skin, respiratory tract, intestines), and is also one of the main causes of late NS in premature infants [31]. Its dominance in our study may be associated with both its role in the development of infections (in most cases) and asymptomatic carriage, which is also a negative prognostic factor [32]. The level of resistance of S. epidermidis to oxacillin that we identified was 93.8%. The high frequency of detection of this pathogen in our study determined the overall high level of resistance of staphylococci to oxacillin. A retrospective analysis of data from newborns with sepsis in Sweden (period 2006–2016) indicates a high level of resistance of S. epidermidis to isoxazolylpenicillins — 91.7% [26]. According to a retrospective descriptive study of medical records of newborns admitted to the NICU (Brazil, 2015–2022, *n*=1610), infections caused by oxacillin-resistant staphylococci occurred in 12%, of which S. epidermidis was responsible for 60.1% [33]. Infections caused by oxacillin-resistant staphylococci, according to Ferreira et al (2024), are associated with prolonged hospitalization (from 10 to 46 days) and increased mortality (from 10.2% to 19.2%). The mean time from infection to death was 15 days (IQR: 8–40) [33]. According to a literature review of studies on NS, Wang et al (2022) found *S. epidermidis* to have the highest resistance to such ABDs as erythromycin and penicillin [29]

The second most frequently detected pathogen identified in our study, *S. haemolyticus*, was also characterized by an extremely high level of resistance to oxacillin, 86.7%. Being a representative of CNS, this MOs typically colonizes the skin. In modern clinical practice, most of *S. haemolyticus* strains exhibit multidrug resistance, which makes them a significant cause of severe NS [34, 35]. Representatives of the ESKAPE group were identified in a third of all cultures in our study 582 (28.6%). The significant role of ESKAPE group MOs in the genesis of infections in newborns is confirmed by many studies. Tzialla et al (2024), having analyzed the global database of outbreaks of nosocomial diseases (Outbreak Database, https://www.outbreak-database.com/Home.aspx), found that the main pathogens in newborns in the NICU were *S. aureus* (24%) and *Klebsiella* spp. (20%) [19].

The characteristics of patients with NS caused by *S. aureus* are well described in the work demonstrating the results of a retrospective study of medical records of patients over a 20-year period (Australia) [36]. The overall incidence was 0.10 per 1000 live births, the analysis found its decrease after 2011 (from 2001 to 2010 — 0.13/1000; from 2011 to 2020 — 0.07/1000). The authors identified EDTA as the main source of biomaterial for cultures that revealed the growth of this pathogen. An important discovery was the detection of a link between *S. aureus* infection and the development of subsequent neurological deterioration [36]. According to our data, the only noteworthy aspect regarding the assessment of the susceptibility parameters of *S. aureus* to ABDs was the detection of resistance to oxacillin in 17.1% of strains and to gentamicin in 9.2%. This differs from the results

obtained by Oldendorff et al (2024) for a population of newborns with sepsis in Sweden: the authors did not find a single case of resistance to isoxazolyl penicillins, nor any significant resistance to other ABDs [26]. An analysis of *S. aureus* antibiotic resistance parameters based on data from patients in a pediatric hospital in Beijing (2013–2022) revealed high levels of resistance to penicillin (89.5%) and erythromycin (73.8%) against the background of high susceptibility to linezolid, vancomycin, rifampicin, and moxifloxacin [37].

The next significant pathogen, ranking fourth in terms of detection frequency in the NICU population in our study, is *K. pneumoniae* (*n* = 210; 10.3%). This pathogen is one of the most common causes of gram-negative NS. According to Nordberg et al (2024), *K. pneumoniae* ranked second after *E. coli*, accounting for 18.7% (20 out of 107) [38]. According to our study, about half of all K. pneumoniae strains were resistant to ceftazidime (48.8%), and 100% demonstrated resistance to ampicillin. This distinguishes our data from the results of Nordberg et al (2024), who did not find significant resistance of this MOs to the tested ABDs [38], but coincides with the data obtained by You et al (2020) in a retrospective analysis of the medical records of newborns with sepsis caused by *K. pneumoniae* in China (the period from 2000 to 2019): the authors found resistance to ampicillin in 98.8%, ceftazidime in 71.5%, cefazolin in 87.2%, and cefotaxime in 82.6%. At the same time, the pathogen retained high sensitivity to aminoglycosides and fluoroquinolones. [39]. The last MO among the top 5 pathogens identified in our study was *E. coli* (*n*=188; 9.2%). In the structure of pathogens of gram-negative NS in Sweden, this MO was identified as the main one (43.9%; 47 out of 107) [38]. The main characteristics of *E. coli* revealed in our study were resistance to ceftazidime in 28.2% of strains, to ampicillin in 64.9% of strains, and to sulfamethoxazole trimethoprim in 28.2%. In this regard, it is interesting to compare our data with results of a retrospective cohort study that included data from medical records of neonates infected by *E. coli* and hospitalized in NICU (USA, period 2009–2017, *n*=733). These results witnessed the highest levels of resistance to ampicillin (99.9%), aminoglycosides (99.7%), carbapenems (91.8%), cefazolin (95.8%), ceftriaxone (91.5%) and sulfamethoxazole trimethoprim (94.2%) [40]. Resistance of E. coli isolated from newborns in hospitals was demonstrated in the results of a multicenter study conducted in China (2021–2022): 75.5% of strains were resistant to cefotaxime, 65.4% to sulfamethoxazole trimethoprim, and 48.4% to ciprofloxacin [41]. The levels of *E. coli* resistance identified in our results are not as significant as in the studies cited above, but nevertheless indicate the risks of antibiotic therapy for neonatal infections caused by this pathogen.

Such MOs, as *A. baumanni* and *P. aeruginosa* were found in our study in 2.3 and 1.1%, respectively. According to the review by Pillay et al (2024), the frequency of NS caused by *A. baumannii* ranges from 1 to 6% [42]. Despite the fact that published data indicate a high level of resistance of this pathogen and the dominance of strains with multidrug resistance [43, 44], our study did not find similar results; the only ABDs to which a decrease in sensitivity was detected were cefepime (17% resistant) and sulfamethoxazole trimethoprim (6.3% resistant). *P. aeruginosa* is one of the MOs with the lowest frequency of detection in our work. In the practice of NICU work, this pathogen is not the leading one; however, its detection indicates the risk of a severe course of the infectious process and is associated with a high frequency of fatal outcomes. Among gram-negative causative agents of NS in Sweden (analysis of 11 years of practice), *P. aeruginosa* was detected in 3.7% of cases (4 out of 107), while resistance to ABDs was not detected [38]. Our data revealed variable levels of *P. aeruginosa* antibiotic resistance. 22.4% of strains were resistant to cefepime, 64.1% had intermediate resistance. Ceftazidime-resistant strains were seen in 30.5% and in 65.8% intermediate resistance was detected. Resistance to meropenem was identified in 20.1% and to amikacin in 19.5%.

### Limitations of the study

Our study included the materials of cultures of neonates hospitalized in the largest specialized clinic of the Russian Federation, however, these data may not reflect the landscape of pathogens typical for all regions of the country. Our study had retrospective design, and we did not assess the effectiveness of the antibiotic therapy. Also, the presented data did not include estimation of clinical outcomes depending on the type of pathogen identified and the parameters of its antibiotic resistance, which will be the next stage of our work.

## CONCLUSION

In the population of neonates hospitalized in the NICU with a mean gestational age of 34.6±4.8 weeks, we found a fairly high frequency of pathogen isolation — 39.3%. The largest number of pathogens was obtained from the pharynx (65.5%), rectum (10.4%) and endotracheal aspirate (8.9%). Gram-positive microflora dominated in the structure of pathogens (59.6%). Analysis of antibiotic resistance parameters revealed a high level of staphylococcal

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resistance to oxacillin (71.2%). ESKAPE group MOs were determined in 28.6%, the leader was *S. aureus* (10.7%, of which 17% of strains were resistant to oxacillin). *K. pneumoniae* was the most common gram-negative MO (10.3%), almost half of the strains were resistant to ceftazidime (48.8%) and 100% — to ampicillin. *E. coli* was the second most frequently determined gram-negative MO identified in neonates in the NICU (9.2%). Resistance to ceftazidime was determined in 28.2% of strains, to ampicillin in 64.9%, and to sulfamethoxazole trimethoprim in 28.2%. *A. baumanni* and *Enterobacter* spp. were characterized by a fairly high sensitivity to all tested ABDs. For *P. aeruginosa*, minimal sensitivity was found to cefepime and ceftazidime (13.5 and 3.7%, respectively). Our results revealed an increase in the levels of antibiotic resistance of pathogens detected in NICU and indicate the importance of dynamic monitoring of the sensitivity of the microflora to the main ABDs used in NICU.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTON

Olga I. Butranova — idea, conducting of research, collection and analysis of data, writing and editing of the draft manuscript; Anastasiia A. Gorbacheva — data collection and analysis, editing of the draft manuscript; Sergey K. Zyryanov — critical revision of the draft manuscript, data collection and analysis of, approval of the final version of the draft manuscript; Oksana G. Ni — conducting of research, data collection and analysis. All authors made an equivalent and equal contribution to the preparation of the publication. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication)

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# Study of acute toxicity, endothelial- and cardioprotective properties of phenolic and thiophenolic derivatives of 2*H*-imidazoles

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**The aim.** To study the acute toxicity, endothelial- and cardioprotective properties of phenolic and thiophenolic derivatives of 2*H*-imidazoles.

**Materials and methods.** The study was performed on white laboratory female BALB/c mice (*n*=57) and male C57Bl/6 mice (*n*=66). Acute toxicity was assessed according to the interstate standard GOST 32644-2014 with histological evaluation of internal organs. Endothelial dysfunction was modeled by 7-day intraperitoneal administration of *N*-nitro-*L*-arginine methyl ester (L-NAME). The studied small molecules were administered intragastrically using a probe. To assess the endothelial protective effect, the levels of systolic and diastolic blood pressure were evaluated, as well as the coefficient of endothelial dysfunction; for the cardioprotective effect, the results of stress tests on the myocardium were evaluated.

**Results.** The study of acute toxicity of the studied small molecules allowed us to classify them as class 4 and 5. The administration of compounds **1(a–d)** and **2(a–c)** to mice at a dose equal to 1/10 of LD50 led to changes in blood pressure and restoration of the dynamics of pharmacological tests in response to the administration of acetylcholine and sodium nitroprusside. Molecules **1b** and **2c** showed statistically significant endothelial protective activity in 3 doses (1/10, 1/50 and 1/100 of LD50). Also, these hit compounds demonstrated cardioprotective effects, recorded by the restoration of the functional capabilities of the myocardium in response to load and in the adrenoreactivity test, and to a lesser extent, during resistance exercise.

**Conclusion**. The studied compounds have low toxicity and have endothelial- and cardioprotective effects. This study may contribute to the formation of an idea about further directions in the study of the pharmacological activity of these molecules from the group of phenolic and thiophenolic derivatives of 2*H*-imidazoles.

**Keywords:** azaheterocyclic compounds; small molecules; endothelial dysfunction; imidazoles; phenols; thiophenols

**Abbreviations:** ED — endothelial dysfunction; CVD — cardiovascular diseases; L-NAME — *N*-nitro-*L*-arginine-methyl ester; $LD_{50}$ — median lethal dose; NO — nitric oxide; SBP — systolic blood pressure; DBP — diastolic blood pressure; CED — coefficient of endothelial dysfunction; ACh — acetylcholine; SNP — sodium nitroprusside.

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# Исследование острой токсичности, эндотелио- и кардиопротективных свойств фенольных и тиофенольных производных 2*H*-имидазолов

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**Цель.** Изучить острую токсичность, эндотелио- и кардиопротективные свойства фенольных и тиофенольных производных 2*H*-имидазолов.

**Материалы и методы.** Исследование выполнено на белых лабораторных мышах-самках линии BALB/c (*n*=57) и на мышах-самцах линии C57Bl/6 (*n*=66). Исследование острой токсичности проводилась по межгосударственному стандарту ГОСТ 32644-2014 с гистологической оценкой внутренних органов. Эндотелиальную дисфункцию моделировали при помощи 7-дневного внутрибрюшинного введения N-нитро-L-аргинин-метиловый эфира (L-NAME). Исследуемые малые молекулы вводились внутрижелудочно с помощью зонда. Для оценки эндотелиопротективного действия оценивали уровни систолического и диастолического артериального давления, а также коэффициент эндотелиальной дисфункции, для кардиопротективного эффекта — результаты нагрузочных проб на миокард.

**Результаты.** Изучение острой токсичности исследуемых малых молекул позволило отнести их к 4 и 5 классу. Введение мышам соединений **1(a–d)** и **2(a–c)** в дозе, равной 1/10 от $LD_{50}$, привело к изменению уровня артериального давления и восстановлению динамики фармакологических проб в ответ на введение ацетилхолина и нитропруссида натрия. Статистически значимую эндотелиопротективную активность в 3 дозах (1/10, 1/50 и 1/100 от $LD_{50}$) показали молекулы **1b** и **2c**. Также данные соединения-хиты продемонстрировали кардиопротективные эффекты, регистрируемые восстановлением функциональных возможностей миокарда в ответ на нагрузку в объёме и в пробе на адренореактивность, и в меньшей степени — при проведении нагрузки сопротивлением.

**Заключение.** Исследуемые соединения имеют низкую токсичность и обладают эндотелио- и кардиопротективным действием. Данное исследование может поспособствовать формированию представления о дальнейших направлениях в изучении фармакологической активности данных молекул из группы фенольных и тиофенольных производных 2*H*-имидазолов.

**Ключевые слова:** азагетероциклические соединения; малые молекулы; эндотелиальная дисфункция; имидазолы; фенолы; тиофенолы

**Список сокращений:** ЭД — эндотелиальная дисфункция; ССЗ — сердечно-сосудистые заболевания; L-NAME — N-нитро-L-аргинин-метиловый эфир; $LD_{50}$ — полулетальная доза; NO — оксид азота; САД — систолическое артериальное давление; ДАД — диастолическое артериальное давление; КЭД — коэффициент эндотелиальной дисфункции; АХ — ацетилхолин; НП — нитропруссид натрия.

## INTRODUCTION

Endothelial dysfunction (ED) makes a significant contribution to the development of socially significant cardiovascular diseases (CVD). The search for effective methods of pharmacological therapy and prevention of this pathology is extremely relevant at present. Various risk factors, such as hypercholesterolemia, hyperhomocysteinemia, hyperglycemia, hypertension, smoking, inflammation, and aging, contribute to the development of ED. The impaired structure and function of the endothelium play the main role in the pathogenesis of diseases such as arterial

hypertension, atherosclerosis, diabetes mellitus, and many others. Any disturbance affecting the equilibrium of the endothelium as a physical barrier, as well as metabolism, synthesis, and release of biologically active substances, can contribute to the development of ED, which leads to the progression of CVD [1, 2]. It is obvious that multiple mechanisms are involved in ED, namely inflammation, increased reactive oxygen species, cell apoptosis, increased production of vasoconstrictors, decreased production of vasodilators, and vascular remodeling. However, a decrease in the bioavailability of nitric oxide (NO) appears to play the central role in the development of this pathology [3, 4].

Researchers around the world are constantly searching for new biologically active molecules to develop medicines based on them for the treatment of a wide range of socially significant diseases, including cardiovascular pathologies. Chemicals used in pharmaceuticals based on small organic molecules are of particular importance.

Small molecules are low molecular weight chemical compounds that have the ability to regulate or affect certain biological processes. Azaheterocyclic six- and five-membered systems rightfully occupy a special place among numerous known substances, on the basis of which a large number of known and effective Chemicals used in pharmaceuticals have been created [5]. Among five-membered molecules, bifunctional derivatives based on imidazoles, modified with various biogenic fragments (amines, carboxyl groups, phenols, indoles, and their other analogs), which are used in the therapy of inflammatory, viral, bacterial, neurodegenerative, and other pathological processes, have recently deserved special attention [6, 7]. At the same time, researchers pay special attention to ensuring that the methods for obtaining these molecules are effective, economical, environmentally friendly, and also comply with the principles of "green chemistry" [8, 9].

It is worth mentioning that at the moment there are no targeted medicines aimed to correct ED. Pleiotropic effects of known medicines are used, which, favorably affect the endothelium together with the main function. Small molecules that simultaneously affect several targets involved in the pathogenesis of ED (multitarget compounds) are the most promising. Taking in account the dominant role of reducing NO bioavailability in the development of ED, small molecules for the treatment of this pathology should be able to restore NO levels in the endothelium [10]. Thus, the synthesis of small molecules that affect various pathways in the pathogenesis of ED development is promissing in modern CVD therapy.

**THE AIM.** To study the acute toxicity, endothelial- and cardioprotective activity in vivo of synthesized small molecules from the group of phenolic and thiophenolic derivatives of 2*H*-imidazoles.

## MATERIALS AND METHODS

Phenolic and thiophenolic derivatives of 2H-imidazoles **1(a–d) and 2(a–c)** were synthesized in accordance with the previously published method [11, 12]. The previously undescribed hydrochloride salt **1b** was isolated and characterized by NMR and IR spectroscopy, mass spectrometry, and elemental analysis data.

### Animals

The study was conducted from November 2023 to April 2024. The acute toxicity was performed on white laboratory BALB/c mice (virgin females) (*n*=57; 3 mice per cage). Pharmacological activity was studied on laboratory male C57Bl/6 mice (*n*=66; 6 mice per cage). Animals were taken into the experiment at the age of 8–10 weeks, weighing 18–22 g, without external signs of disease. All laboratory animals were obtained from the nursery of the Belgorod State National Research University (Belgorod, Russia). After passing a 14-day quarantine regime, the mice were stratified by weight and placed in separate cages. The animals were kept in a standard biologically clean experimental room. Feeding was carried out in accordance with GOST 33216-2014 "Guidelines for the maintenance and care of laboratory animals". The daily diet throughout the study included granulated feed (GOST 50258-92) and filtered water disinfected with UV irradiation. 4 hours before the introduction of the test substances, all experimental animals were subjected to complete food deprivation with free access to water, and feeding was resumed 2 h after the test substance was introduced. On the eve of the day of necropsy at 16:00, the animals were deprived of food with free access to water. The experiments were carried out in compliance with the requirements of Federal Law No. 498-FZ of December 27, 2018 "On responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation", the Rules of Good Laboratory Practice in conducting preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), the Directive of the European Community (86/609 EC), the Rules of International Recommendations of the European Convention for the Protection of Vertebrate Animals Used in Experimental Studies (1997) and the Rules of Good Laboratory Practice (approved by Order of the Ministry of Health of the Russian Federation No. 199n of April 1, 2016).

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All *in vivo* experiments were conducted at the Belgorod State National Research University (Belgorod, Russia). The study was approved by the Commission for the Control of the Maintenance and Use of Laboratory Animals of the Belgorod State National Research University (Belgorod, Russia), expert opinion No. 01-07i/23 dated July 10, 2023.

### Study of acute toxicity of synthesized small molecules

In the study of acute toxicity of seven compounds **1(a–d) and 2(a–c),** the interstate standard GOST 32644-2014 "Methods of testing the effects of chemical products on the human body. Acute oral toxicity — method for determining the class of acute toxicity" (OECD, Test No. 423) was observed. Based on the results of these works, the toxicity class was determined in accordance with the globally harmonized system of hazard classification and labeling of chemical products that cause acute toxicity and the range of doses for subsequent determination of $LD_{50}$. The structural formulas of the studied samples are shown in Figure 1.

At the Federal University named after the First President of Russia B.N. Yeltsin, a preliminary stage of studying the synthesized phenolic and thiophenolic derivatives of 2*H*-imidazoles **1(a–d) and 2(a–c)** was carried out. The results of studies of the acute toxic effect in vitro of these pharmaceutical substances were obtained. Based on the data obtained, a suggestion was made about the low danger of acute toxicity of the substances, which formed the basis for choosing the initial dose — 2000 mg/kg for this study. According to the presented data on the solubility of the studied compounds, obtained during preliminary studies, an aqueous solution of dimethyl sulfoxide (in a ratio of 1:1) was used as a solvent for intragastric administration of the medicines. The studied substances were administered in a volume of 0.05 ml/10 g of weight in one dose using an atraumatic gastric probe. The body weight of the animals was measured and the dose was prepared immediately before the administration of the medicine.

The study of acute toxicity was carried out on BALB/c mice, which were grouped into 3 individuals per cage for each stage of the experiment (*n*=57). Observation of the animals was carried out for 14 days. Within 2 h after the administration of the dose of the medicine, each individual was under individual continuous observation. Then, up to 12 h from the moment of administration of the studied dose, the frequency of individual examination was every 60 min, and then daily in the morning and evening for all 14 days. The observation included an assessment of the general condition with fixation of the clinic of poisoning (changes in the skin and coat, eyes and mucous membranes, assessment of the respiratory, circulatory, autonomic and central nervous systems, as well as somatomotor activity and the nature of behavior) and counting the number of animals that died during the experiment.

Animals that died during the experiment were subjected to autopsy, and survived animals were humanely euthanized at the end of the experiment (overdose of ethyl ether) and subjected to autopsy. The resulting organs and tissues were fixed in 10% buffered formalin. After macroscopic evaluation, histological dissection of the material was carried out, followed by wiring according to the standard scheme using an AGT-11 FMP apparatus (Russia). After wiring, paraffin embedding was performed with the formation of blocks using an MPS/P2 SLEE medical GmbH filling station (Germany). The finished paraffin blocks were subjected to microtomes using an RMD-3000 rotary microtome (Russia). Microtome sections were placed on slides with an adhesive coating, after which standard histological staining was performed on an automated multistainer Histoprocessor TLP-144 (Russia) — hematoxylin + eosin. The stained sections were evaluated using a Nikon Eclipse E200 microscope (Japan).

### Study of the endothelial protective activity of the obtained small synthesized molecules on the model of L-NAME-induced endothelial dysfunction

The animal groups included an intact group (*n*=6, without the introduction of medicines and modeling of pathology), a control group (*n*=6, with modeling of L-NAME-induced ED without pharmacological correction) and groups with L-NAME-induced ED with pharmacological correction with the studied substances (*n* =66; 6 individuals per group, 7 groups in the first stage, 4 additional groups in the second stage).

To model ED, an inducer of endothelial dysfunction was used — a non-selective NO-synthase blocker *N*-nitro-*L*-arginine methyl ester (L-NAME, Sigma Aldrich, USA), which was administered daily 1 time per day intraperitoneally at a dose of 60 mg/kg for a week.

In order to correct ED, the synthesized small molecules **1(a–d)** and **2(a–c)** were administered 1 time per day intragastrically using a probe for small laboratory animals for 7 days. The control group was administered intragastrically with water for injection in a volume of 0.05 mL/10 g of weight. The development of ED in experimental animals, as well as the degree of its correction by the studied medicines, was assessed by dynamic non-invasive measurement of blood

pressure on the tail of rodents, as well as using the calculated coefficient of endothelial dysfunction (CED).

The pressure was measured three times, on the day the animals were removed from the experiment on the "Systola" apparatus from Neurobotics (Moscow, Zelenograd). The hardware and software complex has a built-in pump that automatically pumps pressure into the tail cuff until the blood flow pulsations stop, and then, slowly reducing the pressure, measures the systolic and diastolic values based on the readings of the infrared pulse sensor worn on the animals' tail after the cuff. Previously, the animal was fixed using the Teremok restrainer from Neurobotics (Moscow, Russia), and then heated to a temperature of 32–37°C for 10–15 min, being on the Flogiston heating platform from Neurobotics (Moscow, Russia). This made it possible to ensure blood circulation in the tail vessels in the required volume and stabilize blood flow. The obtained hemodynamic parameters using the Systola software (Version 1.3.1) were reflected in the form of graphs of the recording curve of blood pressure and pulse and were used in the future to evaluate the results of the experiment.

On the 8th day of the experiment, after preliminary anesthesia with zolazepam (Virbac, Russia) 2.5 mg/100 g+xylazine (Biogel (Belarus) 2 mg/100 g intraperitoneally, the blood flow velocity in the carotid artery was measured in mice, followed by intravenous functional tests with acetylcholine (ACh) and sodium nitroprusside (SNP), which are used to assess endothelium-dependent and endothelium-independent vasodilation, respectively. Hemodynamic parameters were measured continuously using a perivascular flowmeter sensor Transonic Systems from BIOPAC Systems (USA). The data obtained were evaluated in the AcqKnowledge program. CED is the ratio of the area of the triangle above the blood flow velocity recovery curve in response to the administration of SNP (S(SNP)) to the area of the triangle above the blood flow velocity recovery curve in response to the administration of ACh, i.e. S(ACh))/CED=(S(SNP))/(S(ACh)) (Fig. 2).

### Study of the cardioprotective activity of the obtained small synthesized molecules

The myocardial contractility after modeling the pathology was carried out in anesthetized mice on controlled respiration. The cavity of the left ventricle was probed with a needle through the apex of the heart and, using the RX104A sensor "Biopac Systems, Inc." (USA) and using the computer program Biopac Systems, Inc. (USA), cardiogeodynamic parameters were recorded (pressure in the left ventricle, heart rate — HR). To assess the functional capabilities of the myocardium in animals, stress tests were performed: with a volume load, a test for adrenoreactivity (intravenous single administration of a solution of adrenaline hydrochloride 1.0–5 mol/L), with a resistance load (clamping the ascending aorta for 30 seconds).

### Statistical processing of results

All the data obtained were subjected to statistical processing using the Microsoft Excel 2010. Using descriptive statistics methods, the data were checked for compliance with the law of normal distribution using the Shapiro–Wilk test. Relative and average values (arithmetic mean [M], median [Me], standard deviation [SD], interquartile range) were also calculated. With a normal distribution, the data were presented as M±SD. Given the normal type of data distribution, Student's test was used to compare two samples. Differences were considered significant at $p$ <0.05.

## RESULTS AND DISCUSSION

### Acute toxicity

In the group receiving 2000 mg/kg of 1a, 30 minutes after the bait with the medicine, non-lethal signs of toxicity were observed with suppression of the general activity of the animals, bilateral ptosis. Pathological symptoms completely regressed by the end of 2 h after acute baiting. During the next 6 days of the observation period, the animals remained mobile, adequately reacted to external stimuli and manipulations with them. However, on the 7th day after baiting, the activity of the animals decreased, the animals refused to eat, there was a progressive decrease in the reaction to external stimuli, a hunched posture, ruffled coat, and foci of alopecia. At the 2nd stage, one lethal outcome was recorded (on the 12th day) after baiting with the drug. Surviving animals remained lethargic and inactive throughout the remaining observation period. Pathoanatomical examination revealed changes in the renal parenchyma in the form of severe granular and hyaline-droplet dystrophy of the epithelium of the convoluted tubules (Fig. 3), also in the liver there was moderate granularity of hepatocytes with blurred hepatic beams and plethoric sinusoids. The histological picture allows us to conclude that the medicine **1a** in high doses has moderate pneumo- and hepatotoxicity, and allows us to classify it as class 5 — $LD_{50}$≈2500 mg/kg.

Compound **1b** at a dose of 2000 mg/kg immediately after administration caused an increase in the general activity of the animals, tachypnea, and phenomena of shortness of breath. After 30 min, motor anxiety was replaced by lethargy, the visible mucous membranes and skin acquired a cyanotic shade, and the coat lost its luster and became ruffled. During the next 2 days, the development of tonic-clonic seizures was observed,

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which caused the death of the animals. Survived animals were lethargic, there was a weak reaction to light, tactile and sound stimuli, there was an increased consumption of water and a reduced consumption of feed. The clinic of acute poisoning was observed for up to 48 hours. After that, the animal returned to active life or died. In the interval of 10–16 h after the administration of the medicines, a peak in the mortality of experimental animals was recorded. With further observation for 14 days, no deaths were recorded.

Pathomorphological examination revealed multiple hemorrhages on the mucous and serous membranes of internal organs, enlargement of the liver, the liver was of flabby consistency, grayish in color on the cut, and a decrease in the airiness of the lungs. Microscopically, in the liver, subtotal disruption of histoarchitectonics was noticeable, hepatic beams were difficult to determine, hepatocytes had signs of total severe balloon dystrophy (Fig. 4). At the same time, focal interstitial fibrosis with plethora and mononuclear infiltration was noted in the lungs. The administration of 300 mg/kg of compound **1b** did not cause pathological symptoms during the entire observation period. According to the results of the autopsy, no pathological changes in the internal organs of the animals in this group were found, the general histological picture corresponded to the results of the control group. Thus, compound **1b** can be classified as toxicity class 4 — $LD_{50}$≈2000 mg/kg, in doses exceeding LD50 1b, it has a pronounced hepatotoxic and moderate pneumotoxic effect.

Upon administration of the maximum dose of substance 1c, initial signs of poisoning appeared after 30 min. The signs of poisoning included a 2–3-minute period of pronounced anxiety, tachypnea, impaired coordination, alternating with a state of general lethargy, decreased motor activity, and suppression of behavioral and exploratory reactions. However, activity recovered within the next 2 days. During dynamic observation over the subsequent 14 days, lethal outcomes were recorded in some animals (on days 7 and 8). A single administration of 300 mg/kg of compound 1c did not cause pathological symptoms during the entire observation period. According to the autopsy results, no pathological changes in the internal organs of animals in this group were found; the overall histological picture corresponded to the results of the control group. Thus, compound **1c** can be classified as toxicity class 4 — $LD_{50}$≈1000 mg/kg.

During the study of compound **1d** at a dose of 2000 mg/kg, immediately after administration, pronounced anxiety of the experimental animals was observed. Within the next 15–20 min, the respiratory rate increased, the mice stood on their hind legs, sometimes supported by the walls of the cage, and retraction of the intercostal spaces during breathing was observed. After 40 min, the excitement was replaced by a state of general lethargy, decreased motor activity, suppression of behavioral and exploratory reactions, and acrocyanosis was observed. Lethal outcomes were recorded in the interval of 3–4 hours after drug administration. Autopsy revealed signs of general acute venous hyperemia, characterized by plasmorrhages and edema, multiple diapedetic hemorrhages, and necrotic changes in parenchymal organs (Fig. 5). Administration of 300 mg/kg of compound 1d did not cause pathological symptoms during the entire observation period. According to the autopsy results, no pathological changes in the internal organs of animals of this group were found; the overall histological picture corresponded to the results of the control group. Thus, compound **1d** can be classified as toxicity class 4 — $LD_{50}$≈500 mg/kg; in doses exceeding $LD_{50}$, **1d** has a toxic effect on the heart muscle, leading to acute cardiovascular failure.

Immediately after administration of compound **2a** at a dose of 2000 mg/kg, an increase in the number of grooming acts was observed in the animals. After 10–15 min, symptoms of conjunctivitis (swelling and hyperemia of the eyelids) were observed. 40 min after medicine administration, the activity of the animals decreased, indicators of orienting-exploratory activity decreased (decrease in the number of stands), and signs of a central depressant effect were observed (decrease in the number of defecation acts). The clinical picture of acute poisoning was observed for up to 24 hours. One lethal outcome was recorded 10 hours after medicine administration. Subsequently, the animal returned to active life. Autopsy revealed no pathological changes in the internal organs of animals in this group; the overall histological picture corresponded to the results of the control group. Thus, compound **2a** belongs to toxicity class 5 — $LD_{50}$≈2500 mg/kg. Administration of high doses of **2a** close to LD50 has a toxic effect on the central nervous system, with suppression of locomotor and orienting-exploratory activity.

In the group receiving 2000 mg/kg of **2b**, 30 min after medicine administration, non-lethal signs of toxicity were observed with suppression of locomotor and orienting-exploratory activity, as well as the development of postural-kinetic tremor. Pathological symptoms completely regressed by the end of the first day. Throughout the entire subsequent observation period, the animals remained mobile, adequately reacted to external stimuli and manipulations with them, and no lethal outcomes were recorded. Pathological examination of the stomach preparations revealed chronic active inflammation in the base of

the lamina propria and in the submucosal layer (Fig. 6); otherwise, the autopsy results did not differ from the control group, which indicates a local irritant effect of compound **2b** and low overall toxicity and allows it to be classified as class 5 — $LD_{50}$≈5000 mg/kg.

A single intragastric administration of 2000 mg/kg of 2c after 40–45 min led to a decrease in the locomotor activity of the animals, lethargy, the coat became ruffled, and an increase in the frequency of respiratory acts was noted. By the end of the first day, the visible skin and mucous membranes acquired a yellowish tint. In the interval of 20–24 hours after medicine administration, the peak of the mortality of experimental animals was recorded. Survived animals remained lethargic and inactive for 2–3 days after acute administration; some animals remained lethargic throughout the entire observation period. Autopsy revealed jaundice of the serous and mucous membranes of internal organs, an increase in the size and density of the liver, and the liver had a grayish color on the cut. Histological examination revealed a subtotal disruption of the liver histoarchitecture; the hepatic trabeculae were sharply smoothed, and the boundaries between hepatocytes were practically not defined. Hepatocytes were characterized by extreme polymorphism with pronounced balloon dystrophy; nuclei with dense chromatin were found, as well as pyknotic nuclei. In addition, there was heterogeneous interstitial mononuclear infiltration, as well as moderate hyperemia. After administration of 300 mg/kg of 2c, signs of toxicity with suppression of general activity were observed after 40 minutes. 1 lethal outcome was recorded at the first stage of the study after 36 hours of administration.

The activity of survived animals completely recovered on days 5–6. Pathomorphological examination of the dead mouse revealed signs of toxic liver damage, while surviving animals showed a pronounced disruption of the histoarchitecture of the liver tissue with centrilobular hyperemia, without signs of necrosis (Fig. 7). Based on the results of the study, it can be concluded that the drug has pronounced hepatotoxicity and can be classified as class 4 — $LD_{50}$≈1000 mg/kg.

The results of assessing the toxicity class of the studied compounds are presented in Table 1.

### Evaluation of the Endothelioprotective Activity of Small Synthesized Molecules on a Model of L-NAME-Induced Endothelial Dysfunction

Modeling of ED induced by L-NAME administration contributed to the formation of arterial hypertension and an increase in systolic blood pressure (SBP) by more than 1.35 times (Table 2), which led to a change in pharmacological tests in response to the administration of ACh and NP. The development of these changes was evidenced by a statistically significant ($p$ <0.05) increase in CED by more than 4 times compared to intact animals.

Administration of compounds **1(a–d) and 2(a–c)** to sexually mature male mice at doses equal to 1/10 of LD50 led to a change in blood pressure and restoration of the dynamics of pharmacological tests in response to the administration of ACh and NP. This dose was chosen because it corresponds to the generally accepted practice in screening studies of the pharmacological activity of new compounds[1]. A statistically significant difference in the reduction of blood pressure levels was observed for compounds with laboratory codes **1b and 2c** ($p$ <0.05). A comparable dynamic was also noted for the CED indicator.

At the next stage, a preliminary assessment of the effectiveness of 2 hit compounds (the most active compounds) was carried out in 3 doses: the first dose corresponded to 1/10 of LD50, the second dose — 1/50 of LD50, and the third — 1/100 of LD50. The choice of these ranges is due to the chemical structure of the substance, the peculiarities of studying the pharmacological activity of compounds, as well as the need, when introducing them into clinical practice, for long-term administration of drugs based on the studied compounds[2]. The studied doses for each compound are indicated in Table 3.

The results of this series of experiments demonstrated high endothelioprotective activity of compounds **1b and 2c** at a dose of 1/10 and 1/50 of LD50 according to the dynamics of SBP and DBP levels (p <0.05), as well as according to the CED indicator at a dose of 1/10 and 1/100 of LD50 for compound 1b and at a dose of 1/10 of LD50 for compound **2c** (p <0.05; Table 4).

Based on the results of these data on the CED indicator, LD50 was calculated for each of the hit compounds during experimental modeling of L-NAME-induced NO deficiency (according to the method of B.M. Shtabsky): for EAH-165 — 86.67 mg/kg, for EAH-280 — 28.33 mg/kg [13].

[1] Karkishchenko NN, Karkishchenko VN, Shustov EB, Kapanadze GD, Revyakin AO, Semenov HH, Bolotova VT., Dulya MS. Methodological recommendations Biomedical (preclinical) study of antihypoxic activity of drugs. Moscow: Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency (Svetly Gory); 2017. 97 p.
[2] Ibid.

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*Investigated azaheterocyclic compounds of the 2H-imidazole series*

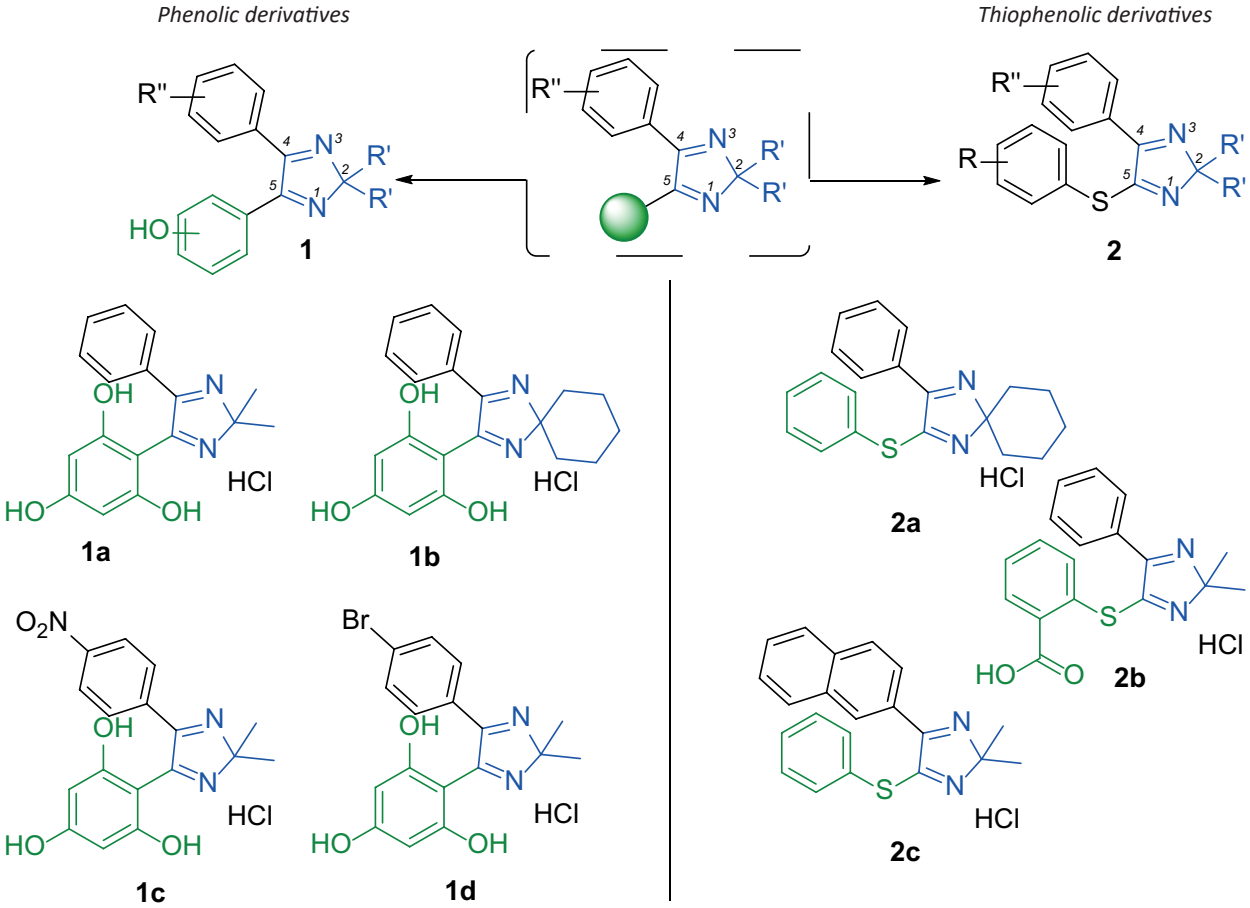


**Figure 1 – Structures of the studied phenolic and thiophenolic derivatives of 2H-imidazole.**

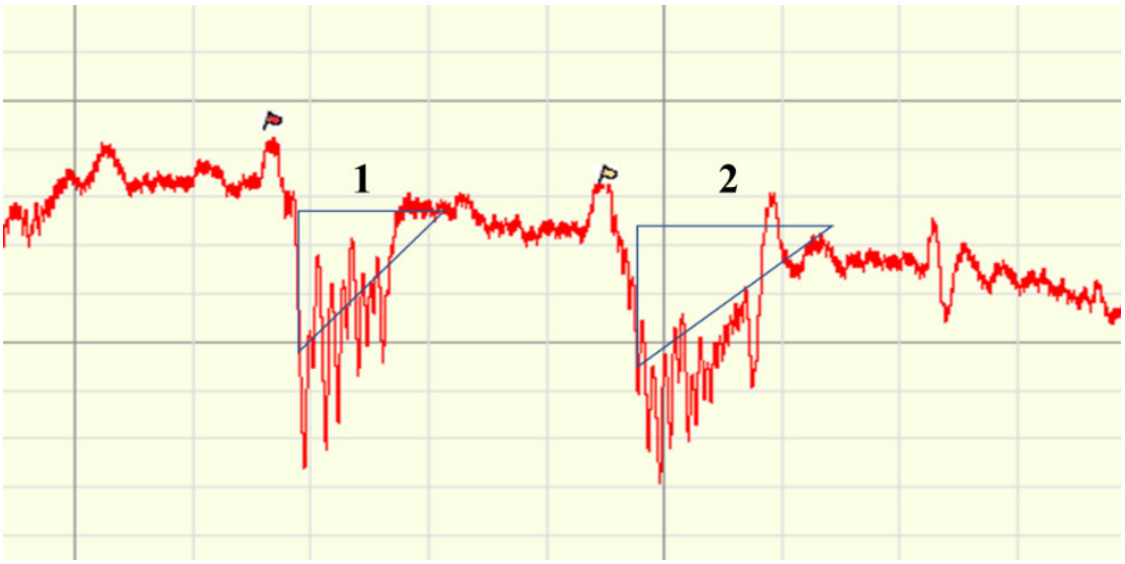


**Figure 2 – Dynamics of blood flow velocity in determining the coefficient of endothelial dysfunction in intact animals.**

Note: 1 — with the introduction of acetylcholine, 2 — with the introduction of sodium nitroprusside.

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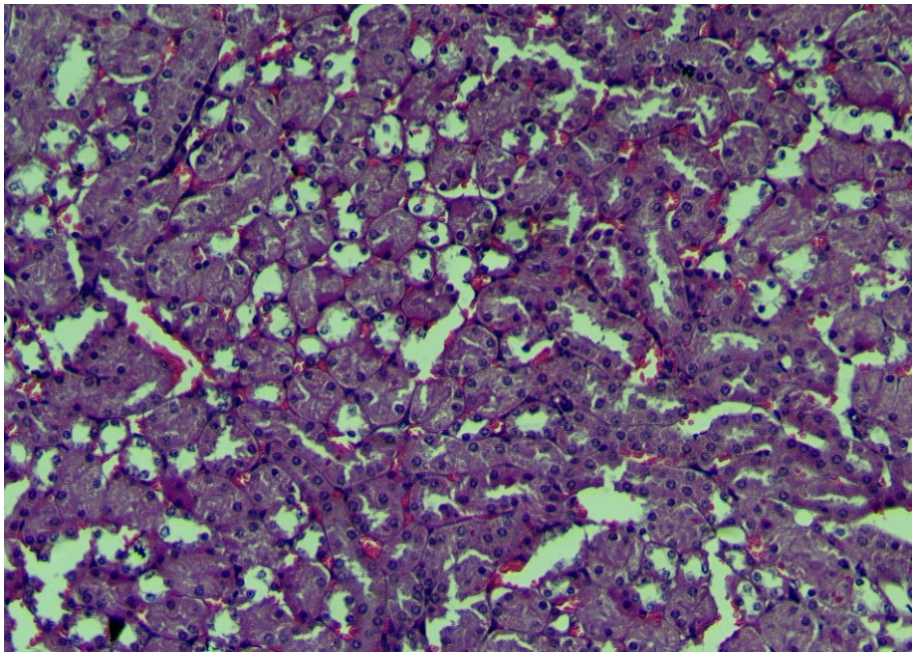

**Figure 3 – Histological structure of the renal parenchyma of a mouse after oral administration of 1a at a dosage of 2000 μg/kg. Staining with hematoxylin+eosin, ×200.**

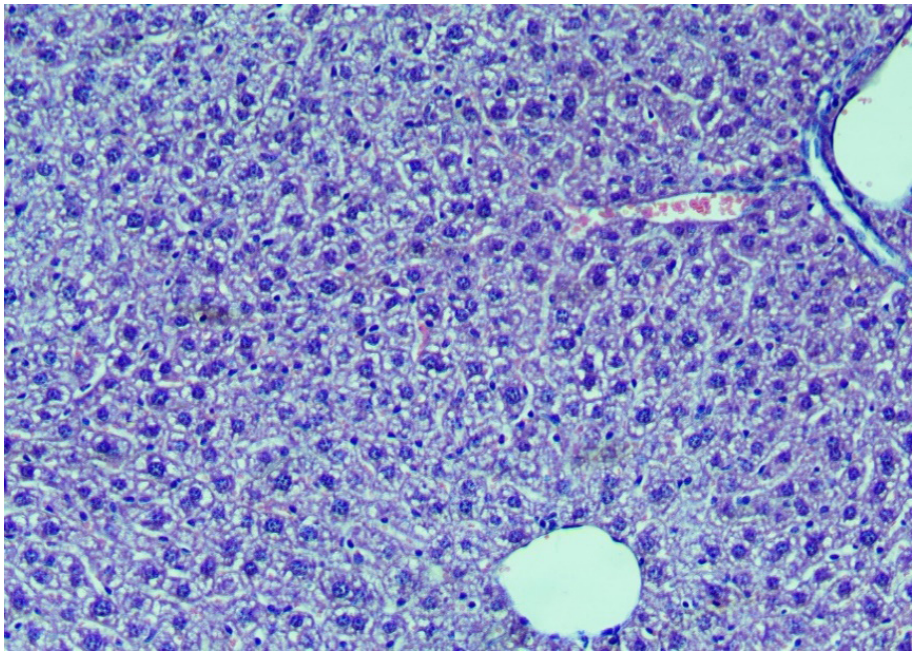

**Figure 4 – Histological structure of the liver of a mouse after oral administration of 1b at a dosage of 2000 μg/kg. Staining with hematoxylin+eosin, ×200.**

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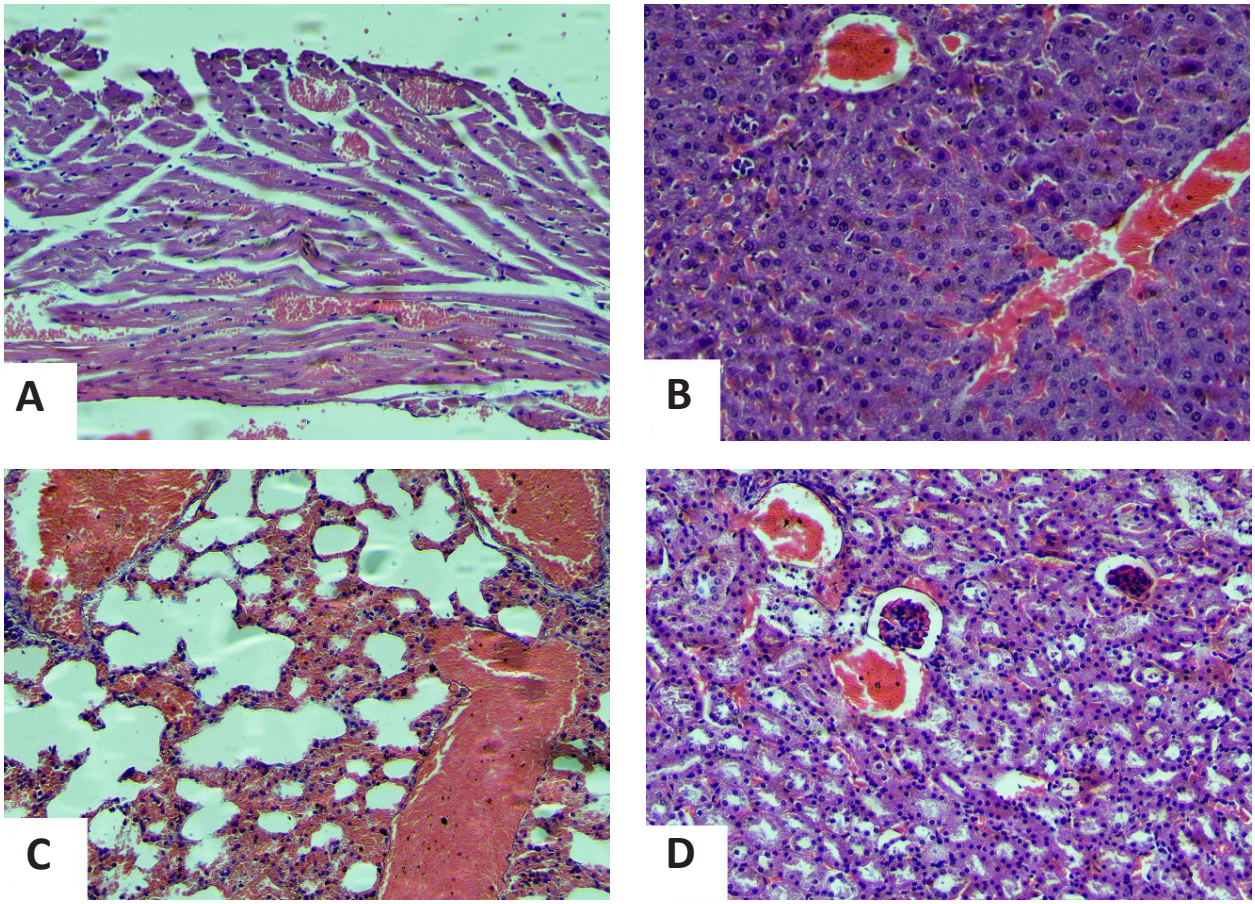


**Figure 5 – Venous hyperemia in the parenchymal organs of a mouse after oral administration of 1d at a dosage of 2000 mcg/kg. Hematoxylin+eosin staining, ×200.**
Note: A — myocardium; B — liver; C — lung; D — kidney.

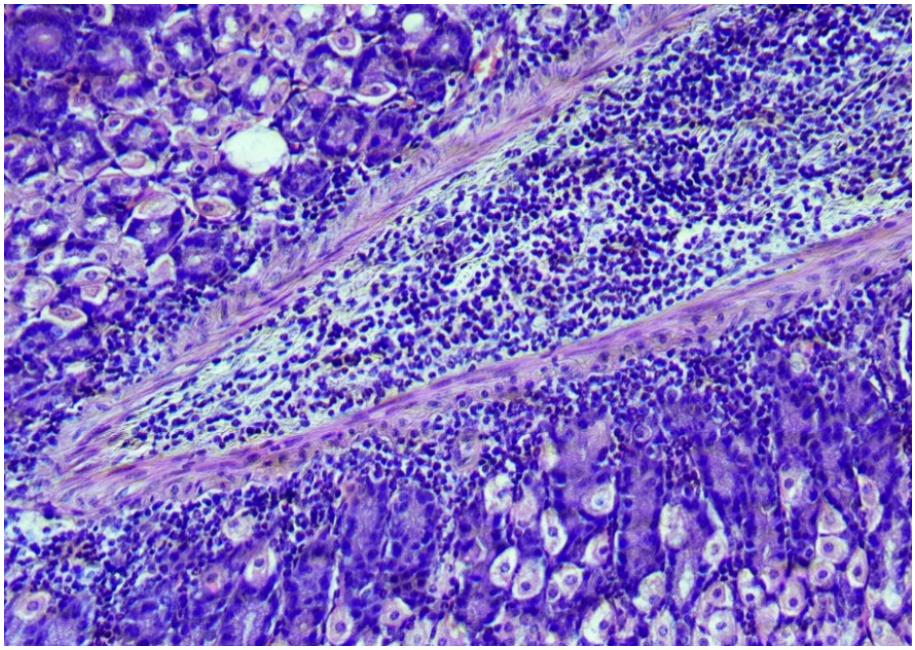

**Figure 6 – Histological structure of the stomach wall of a mouse after oral administration of 2b at a dosage of 2000 mcg/kg. Hematoxylin+eosin staining, ×200.**

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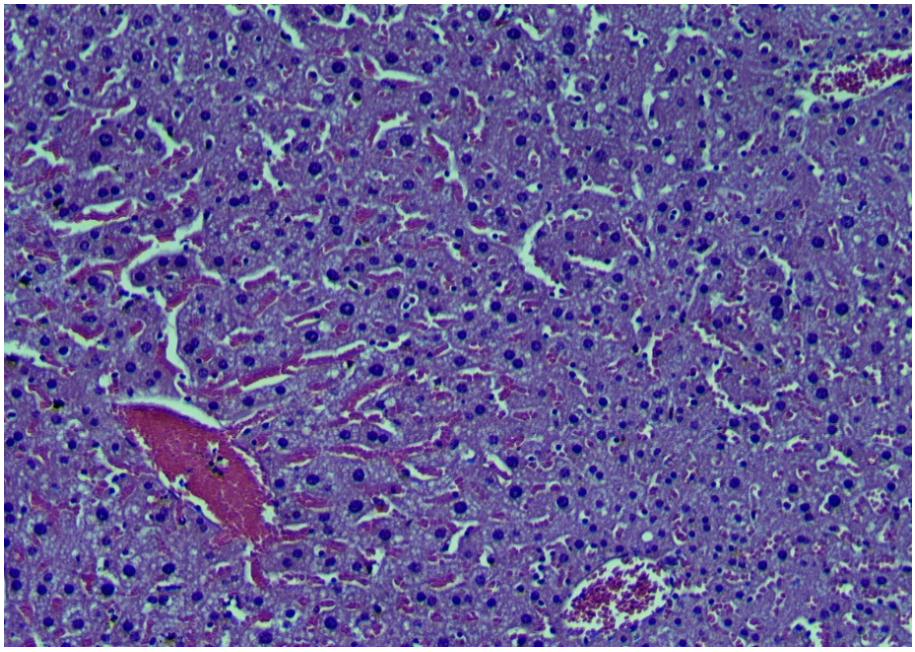

**Figure 7 – Histological structure of the liver of a mouse after oral administration of 2c at a dosage of 2000 mcg/kg. Hematoxylin+eosin staining, ×200.**

**Table 1 – Results of assessing the toxicity class of the studied compounds**

| Compound code | Number of dead animals | | | Toxicity class | $LD_{50}$ value |
|---|---|---|---|---|---|
| | 2000 mg/kg | 300 mg/kg | 50 mg/kg | | |
| 1a | 0/1 | – | – | class 5: 2000–5000 mg/kg | 2500 mg/kg |
| 1b | 1/2 | 0/0 | – | class 4: 300–2000 mg/kg | 2000 mg/kg |
| 1c | 2 | 0/0 | – | class 4: 300–2000 mg/kg | 1000 mg/kg |
| 1d | 3 | 0/0 | – | class 4: 300–2000 mg/kg | 500 mg/kg |
| 2a | 1/0 | – | – | class 5: 2000–5000 mg/kg | 2500 mg/kg |
| 2b | 0/0 | – | – | class 5 or not classified >2000 mg/kg 5000 mg/kg | 5000 mg/kg |
| 2c | 2 | 1/0 | – | class 4: 300–2000 mg/kg 1000 mg/kg | 1000 mg/kg |

**Table 2 – Systolic and diastolic blood pressure and endothelial dysfunction coefficient in experimental groups (M±SD)**

| Group (n=6) | SBP, mm Hg | DBP, mm Hg | CED |
|---|---|---|---|
| Intact | 106.5±3.5 | 64.3±3.3 | 1.01±0.1 |
| L-NAME | 147.5±4.2 | 89.5±3.8* | 4.15±0.5* |
| L-NAME+1a | 137.3±9.7* | 84.5±6.0* | 3.38±1.1* |
| L-NAME+1b | 118.7±7.7** | 72.8±5.1** | 1.47±0.4** |
| L-NAME+1c | 132.8±9.39* | 83.8±6.3* | 2.90±0.8* |
| L-NAME+1d | 136.3±8.4* | 83.2±11.5* | 3.57±0.7* |
| L-NAME+2a | 129.2±8.6* | 83.2±7.9* | 2.87±0.9* |
| L-NAME+2b | 130.5±8.4* | 85.5±6.3* | 3.31±0.9* |
| L-NAME+2c | 119.8±8.2** | 72.2±6.1** | 1.85±0.6** |

Notes: SBP — systolic blood pressure; DBP — diastolic blood pressure. * $p$ <0.05 when compared with the group of intact animals; ** $p$ <0.05 when compared with the L-NAME group.

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**Table 3 – Range of doses of the studied compounds for conducting studies of pharmacological activity on animal models of endothelium-associated pathology**

| Compound code | Preliminary $LD_{50}$ value | 1/10 of $LD_{50}$ | 1/50 of $LD_{50}$ | 1/100 of $LD_{50}$ |
|---|---|---|---|---|
| **1a** | 2500 mg/kg | 250 mg/kg | – | – |
| **1b** | 2000 mg/kg | 200 mg/kg | 40 mg/kg | 20 mg/kg |
| **1c** | 1000 mg/kg | 100 mg/kg | – | – |
| **1d** | 500 mg/kg | 50 mg/kg | – | – |
| **2a** | 2500 mg/kg | 250 mg/kg | – | – |
| **2b** | 5000 mg/kg | 500 mg/kg | – | – |
| **2c** | 1000 mg/kg | 100 mg/kg | 20 mg/kg | 10 mg/kg |

Notes: * $p$ <0.05 when compared with the group of intact animals; ** $p$ <0.05 when compared with the L-NAME group.

**Table 4 – Systolic and diastolic blood pressure and CED in experimental groups of hit compounds (M±m)**

| Group ($n$=6) | SBP, mm Hg | DBP, mm Hg | CED |
|---|---|---|---|
| Intact | 106.5±3.5 | 64.3±3.3 | 1.01±0.1 |
| *L*-NAME | 147.5±4.2* | 89.5±3.8* | 4.15±0.5* |
| *L*-NAME + 1b, 200 mg/kg | 118.7±7.7** | 72.8±5.1** | 1.47±0.4** |
| *L*-NAME + 1b, 40 mg/kg | 119.2±6.4** | 72.8±4.7** | 1.6±0.4* |
| *L*-NAME + 1b, 20 mg/kg | 124.2±6.1* | 79.6±8.3* | 2.3±0.6*. ** |
| *L*-NAME + 2c, 100 mg/kg | 119.8±8.2** | 72.2±6.1** | 1.85±0.6** |
| *L*-NAME + 2c, 20 mg/kg | 121.2±7** | 77±7.9** | 1.6±0.5* |
| *L*-NAME + 2c, 10 mg/kg | 130.2±8.3* | 79.2±3.7* | 2.8±0.7* |

Notes: SBP — systolic blood pressure; DBP — diastolic blood pressure. * $p$ <0.05 when compared with the group of intact animals; ** $p$ <0.05 when compared with the L-NAME group.

**Table 5 – Cardioprotective effects of the studied small molecules during stress tests in experimental groups**

| Group ($n$=6) | Volume load, mm Hg | Adrenoreactivity, mm Hg | Resistance load, % |
|---|---|---|---|
| Intact | 140.3±6.3 | 199.70±4.7 | 87.3±4.5 |
| *L*-NAME | 203.9±7.2* | 254.65±8.5* | 65.0±5.5* |
| *L*-NAME + 1b, 200 mg/kg | 165.9±8.6*** | 216.63±10.7** | 83.1±6.7 |
| *L*-NAME + 1b, 40 mg/kg | 170.3±7.2*** | 214.82±10.5** | 79.1±6.3 |
| *L*-NAME + 1b, 20 mg/kg | 192.5±11.5* | 225.38±8.9*** | 73.1±8 |
| *L*-NAME + 2c, mg/kg | 160.6±6.4*** | 207.65±9.2** | 81.4±6.1 |
| *L*-NAME + 2c, 20 mg/kg | 157.9±5.7** | 210.58±10.7** | 84.3±4.4** |
| *L*-NAME + 2c, 10 mg/kg | 186.2±2.6*** | 231.27±10.7* | 76.8±4.8 |

Notes: * $p$ <0.05 when compared with the group of intact animals; ** $p$ <0.05 when compared with the L-NAME group.

### Evaluation of the Cardioprotective Activity of Small Synthesized Molecules

Cardioprotective effects were evaluated for hit compounds **1b and 2c** in mice with L-NAME-induced ED. Stress tests were performed and SBP in the left ventricle and heart rate were evaluated. With L-NAME-induced ED, the functional capabilities of the myocardium decreased in animals, as evidenced by a more pronounced increase in SBP in the left ventricle during volume overload — 203.9±7.2 mm Hg in the control group with L-NAME administration; 140.3±6.3 mm Hg in the intact group ($p$ <0.05). During the adrenoreactivity test, a statistically significant increase in left ventricular pressure was also recorded during ED modeling — 254.65±8.5 mm Hg in the control group with L-NAME administration; 199.70±4.7 mm Hg in the intact group ($p$ <0.05). The resistance load test demonstrated a statistically significant decrease in myocardial contractility from 5 to 25 seconds of aortic clamping in the group with L-NAME-induced ED modeling — 65.0±5.5%, compared with the intact group — 87.3±4.5% ($p$ <0.05).

Compounds **1b and 2c** demonstrated cardioprotective effects, recorded primarily by increasing myocardial reserve, restoring the functional capabilities of the myocardium in response to volume load and in the adrenoreactivity test, and to a lesser extent — during the resistance load test. Compound **1b** showed statistically significant results in 2 doses — 1/10 of and 1/50 of $LD_{50}$ in the volume load test,

and in all 3 studied doses in the adrenoreactivity test compared with the L-NAME group, significantly reducing pressure in the left ventricle ($p$ <0.05). Substance **2c** demonstrated a statistically significant cardioprotective effect in the volume load test in all 3 studied doses and in 2 doses — 1/10 and 1/50 of $LD_{50}$ in the adrenoreactivity test, reducing left ventricular pressure ($p$ <0.05). In the resistance load test, only compound **2c** at a dose of 1/50 of $LD_{50}$ significantly increased the frequency of myocardial contractility from 5 to 25 seconds of aortic clamping (Table 5).

Over the past decades, pharmacological interventions for these pathologies have advanced significantly. However, clinical treatment of CVD is still quite complex, as there is no recognized method for improving the condition of the entire vascular bed. The development of medicines is inextricably linked with the constant search, improvement of existing, as well as the development of new methods and approaches for the directed design of molecules [14, 15].

In this study, we demonstrated the endothelioprotective and cardioprotective activity of new synthesized small molecules from the group of azageterocyclic compounds, the presumed mechanism of which is the effect on the synthesis or bioavailability of NO in the vascular bed. Examples of targets that participate in NO metabolism include NOS (NO synthase) [16–18], arginase [19–21], DDAH (dimethylarginine dimethylaminohydrolase) [22, 23], VEGFR2 (vascular endothelial growth factor type 2), PDE5 (phosphodiesterase type 5) [24, 25], B2AR (beta2-adrenergic receptor), ECE1 (endothelin-converting enzyme) [26], AT1 and AT2 (angiotensin 1 and 2 receptors) [27, 28], BH4 [29]. Thus, there is an increasing interest in the search for new and the study of known biomarkers and therapeutic strategies for the prevention and correction of ED. The synthesis of small molecules that affect various pathways in the pathogenesis of ED development is promising in modern pharmacology of CVD.

### Limitations of the study

This study was performed on one model of L-NAME-induced ED. To determine the exact mechanism of action of the studied substances, further study of their pharmacological activity on other ED models is required.

## CONCLUSION

Synthesized small molecules from the group of phenolic and thiophenolic derivatives of 2H-imidazoles under codes **1(b–d) and 2c** belong to toxicity class 4, and **1a and 2(a, b)** — to class 5. The results of this series of experiments demonstrated that the presented molecules have endothelioprotective properties at a dose of 1/10 of LD50. The most active on the L-NAME-induced ED model were substances **1b and 2c**, which in 3 doses (1/10, 1/50 and 1/100 of LD50) showed not only statistically significant endothelioprotective, but also cardioprotective activity. The conducted study will allow us to form ideas about further directions in order to chemically synthesize and subsequently use small molecules from the group of azageterocyclic compounds. The possibilities and prospects of using synthesized small molecules from the group of azageterocycles in cardiovascular pathologies caused by ED is a promising area of pharmacy and medicine.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

## AUTHORS CONTRIBUTION

Olesya A. Puchenkova — participation in the study of acute toxicity, organ harvesting for micro- and macroscopic study, modeling of endothelial dysfunction and carrying out loading myocardial tests, writing the article; Olesya V. Shheblykina — participation in the study of acute toxicity, organ harvesting for micro- and macroscopic study, modeling of endothelial dysfunction and carrying out loading myocardial tests, statistical analysis of results; Daria A. Kostina — modeling of endothelial dysfunction and conducting stress myocardial tests; Anton A. Bolgov — participation in the study of acute toxicity, histological study; Petr R. Lebedev — modeling of endothelial dysfunction and carrying out stress myocardial tests, formalizing the list of references; Vladimir V. Molchanov — animal care, preparation of experimental groups, administration of drugs; Tatyana G. Pokrovskaya — consultation on individual stages of experimental work, ensuring the quality of research; Mikhail V. Korokin — idea, research planning, consultation on individual stages of experimental work, ensuring the quality of research; Egor A. Nikiforov, Nailya F. Vaskina, Tair A. Idrisov — synthesis of experimental substances; Timofey D. Moseev, Vsevolod V. Melekhin — synthesis of experimental substances, writing an article; Mikhail V. Varaksin, Valery N. Charushin, Oleg N. Chupakhin — synthesis of experimental substances, consultation on individual stages of experimental work. All authors confirm their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the research, and preparation of the article, read and approved the final version before publication).

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# Antimicrobial activity of aqueous-alcoholic extracts from myrtle leaves in relation to strains isolated from patients with cystic fibrosis

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The search for new antimicrobial medicines based on medicinal plant raw materials (MPRM) and its effective and safe use in modern pharmaceutical practice remains one of the most pressing issues in pharmacy. Today, the search for new biologically active compounds (BACs) with antimicrobial and antifungal activity is ongoing. Due to the content of the BACs complex, preparations based on MPRMs have a milder effect on the human body compared to synthetic analogues. According to the results of studying some foreign studies and publications on the topic of antimicrobial and antifungal activity, a promising source of BACs, namely the leaves of common myrtle (*Myrtus communis* L.), is of scientific interest.

**The aim**. Analysis and comparative study of the antibacterial activity of samples of extracts obtained using ethanol of various concentrations, and an infusion of common myrtle leaves (*Myrtus communis* L.) against clinical strains isolated from patients with cystic fibrosis.

**Materials and methods.** The objects of the study were water-alcohol extracts from common myrtle leaves, comparison preparations — ethanol with a concentration of 40, 70, 96% and eucalyptus tincture. 5 strains of pathogenic microorganisms isolated from the sputum of patients with cystic fibrosis were used as test cultures. The minimum inhibitory concentration was assessed using the method of double serial dilutions in broth.

**Results.** All water-alcohol extracts from common myrtle leaves showed antimicrobial activity exceeding the control samples against 3 mucoid strains — *Burkholderia cenocepacia*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*. No antimicrobial activity was detected for the remaining 2 strains. A pronounced antimicrobial effect was possessed by 70% tincture and aqueous infusion of leaves.

**Conclusion.** The data obtained during the study allow us to draw conclusions about the further prospects of studying 70% myrtle tincture and aqueous infusion for use in the therapy of patients with cystic fibrosis.

**Keywords:** common myrtle; *Myrtus communis* L.; leaves; cystic fibrosis; antimicrobial activity; tincture; infusion

**Abbreviations:** ABDs — antibacterial drugs; BACs — biologically active compounds; SPh RF — State Pharmacopoeia of the Russian Federation; MPRM — medicinal plant raw material; MBC — minimum bactericidal concentration; MIC — minimum inhibitory concentration.

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# Антимикробная активность водно-спиртовых извлечений из листьев мирта обыкновенного в отношении штаммов, выделенных от пациентов с муковисцидозом

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Поиск новых антимикробных препаратов на основе лекарственного растительного сырья (ЛРС) и его эффективное и безопасное использование в современной фармацевтической практике остается по-прежнему одним из актуальных вопросов фармации. На сегодняшний день постоянно ведётся поиск новых биологически активных соединений (БАС) с антимикробной и противогрибковой активностью. Благодаря содержанию комплекса БАС препараты на основе ЛРС оказывают более мягкое действие на организм человека по сравнению с синтетическими аналогами. По результатам изучения некоторых зарубежных исследований и публикаций на тему антимикробной и противогрибковой активности, научный интерес представляет перспективный источник получения БАС, а именно листья мирта обыкновенного *(Myrtus communis* L.).

**Цель.** Анализ и сравнительное изучение антибактериальной активности образцов извлечений, полученных с помощью спирта этилового различной концентрации, и настоя из листьев мирта обыкновенного (*Myrtus communis* L.) в отношении клинических штаммов, выделенных от пациентов с муковисцидозом.

**Материалы и методы.** Объектами исследования были водно-спиртовые извлечения из листьев мирта обыкновенного, препараты сравнения — спирт этиловый с концентрацией 40, 70, 96% и настойка эвкалипта. В качестве тестовых культур использовали 5 штаммов патогенных микроорганизмов, выделенных из мокроты пациентов с муковисцидозом. Минимальную ингибирующую концентрацию оценивали с помощью метода двойных серийных разведений в бульоне.

**Результаты.** Все водно-спиртовые извлечения из листьев мирта обыкновенного проявляли антимикробную активность, превосходящую контрольные образцы в отношении 3 мукоидных штаммов — *Burkholderia cenocepacia*, *Stenotrophomonas maltophilia* и *Pseudomonas aeruginosa*. По оставшимся 2 штаммам антимикробная активность не выявлена. Выраженным антимикробным эффектом обладала настойка мирта 70% и водный настой листьев.

**Заключение.** Полученные в ходе проведённого исследования данные позволяют сделать выводы о дальнейших перспективах изучения настойки мирта 70% и водного настоя листьев мирта для использования в терапии пациентов, больных муковисцидозом.

**Ключевые слова:** мирт обыкновенный; *Myrtus communis* L.; листья; муковисцидоз; антимикробная активность; настойка; настой

**Список сокращений**: АБЛП — антибактериальные лекарственные препараты; БАС — биологически активные соединения; ГФ РФ — Государственная фармакопея Российской Федерации; ЛРС — лекарственное растительное сырьё; МБК — минимальная бактерицидная концентрация; МИК — минимальная ингибирующая концентрация

## INTRODUCTION

Patients with cystic fibrosis occupy a special place in the category of patients who need antimicrobial therapy on an ongoing basis. Most bacterial strains isolated from patients with cystic fibrosis are characterised by pronounced antibiotic resistance, both genetically mediated and acquired. At the same time, more than 85% of the strains are resistant to one or more antibacterial drugs (ABDs) [1]. Thus, science is faces the task of improving existing antimicrobial drugs and searching for new potential molecules and biologically active compounds (BACs), including to improve the effectiveness of cystic fibrosis therapy and the quality of life of patients with this diagnosis.

According to the international register of the Cystic Fibrosis Foundation[1] (Washington, USA), it has been established that patients with cystic fibrosis are predominantly dominated by microbial flora such as Pseudomonas aeruginosa and Staphylococcus aureus, to a lesser extent — *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cenocepacia* [2, 3]. The main difficulty in the treatment of cystic fibrosis is represented by patients infected with *Burkholderia cenocepacia* strains, which are resistant to most of the ABDs used in modern therapy [4, 5]. In this regard, a search is currently underway for new BACs with antimicrobial and antifungal activity. Due to the content of the BACs complex, drugs based on MPRM have a milder effect on the human body and do not cause addictive syndrome compared to synthetic analogues [6]. It is known that BACs such as flavonoids play a leading role in the formation of the most important pharmacotherapeutic effects of medicinal plants, including antimicrobial, anti-inflammatory, antifungal, antiviral, choleretic, antispasmodic, etc. [7].

Based to the results of studying some domestic and foreign studies and publications on the aim of antimicrobial and antifungal activity of MPRM, such a promising source of BACs as common myrtle (*Myrtus communis* L.) is of scientific interest [8–10]. Due to the presence of flavonoids in the raw materials of myrtle (*Myrtus communis* L.), leaves and fruits, as well as essential oil from this plant, have long been used in many countries as an antimicrobial, antifungal and anti-inflammatory agent [11].

Common myrtle is an evergreen perennial shrub up to 3 m tall, the crown is dense, multi–branched; young shoots are tetrahedral, greenish gray; 2–3-year-old shoots are rounded or slightly faceted, gray or gray–brown. The leaves are glandular opposite, ovate to lanceolate, 2–5 cm long, 1–2.5 cm wide, pointed, whole-edged, leathery, fragrant when rubbed. The flowers are white, arranged one at a time on short pedicels. Blooms in late May and all of June. The fruit is a bluish black (sometimes white), multi-seeded berry, 10–12 mm long and 5–6 mm wide. There are 5–15 seeds in each fruit. The fruits look like round white berries, have a spicy sweet taste and ripen in November–December [12]. It grows in subtropical countries: South America, North Africa, Southern Europe, northwest India, Australia, the Middle East and Western Asia [13]. The *Myrtaceae* family includes 100 genera and 3 000 species. In the Russian Federation, common myrtle grows and is cultivated on the territory of the Republic of Crimea (Nikitsky Botanical Garden, Yalta), as well as in the Caucasus and Krasnodar Territory [14].

Currently, myrtle is not a pharmacopoeial plant in Russia, although it is included in foreign pharmacopoeias[2], [3]. Moreover, the presence of antimicrobial and antifungal effects of extracts from the leaves of this plant encourage Russian scientists to actively explore the possibilities of using this type of MPRM in medical practice.

**THE AIM.** An analysis and comparative study of the antibacterial activity of samples of extracts obtained with ethanol of various concentrations and infusion of myrtle leaves (*Myrtus communis* L.) against to clinical strains isolated from patients with cystic fibrosis.

## MATERIALS AND METHODS

Water-alcohol extracts from myrtle leaves based on ethanol of the chemically pure category (concentrations of 40, 70 and 96%) in the ratio "raw material–extractant" — 1:5 were used as objects of study. Common myrtle leaves were harvested and dried at the Order of the Red Banner of Labor Nikitsky Botanical Garden – National Scientific Center (NBG-NSC) in July 2022 in Yalta, Republic of Crimea, Russian Federation, provided under a scientific cooperation agreement with the Department of Pharmacognosy with the Basics of Phytotherapy of the Samara State Medical University. The raw materials were dried in the air without direct sunlight. The species specificity of the object was confirmed with the help of relevant scientific papers [15–17], as well as herbarium specimens from the herbarium fund of the NBG-NSC[4].

Preparations for comparison and control samples with established antimicrobial activity were ethanol of analytical grade in several main concentrations (40, 70 and 96%) and tincture of *Eucalyptus viminalis* L. 70% produced by Tula Pharmaceutical Factory LLC (series 21112), Russia. To prepare solutions of ethanol 40 and 70%, ethanol 96%, Hippocrates LLC (series 380221), Russia, was used.

The following strains of pathogenic microorganisms isolated from the sputum of patients with cystic fibrosis were used as test cultures: *Pseudomonas aeruginosa* (strain 1), *Pseudomonas aeruginosa* (mucoid strain 2), *Stenotrophomonas maltophilia, Burkholderia cenocepacia* and *Chryseobacterium indologenes.*

The strains of microorganisms included in the

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study were obtained by experienced scientists of the Microbiological Department of the Clinical Diagnostic Laboratory of the Samara State Medical University clinics in accordance with the conclusion of the Bioethics Committee at the Samara State Medical University (extract from Protocol No. 204 of December 11, 2019). The patients underwent microbiological examination on an outpatient basis in accordance with the National Clinical Guidelines of the Russian Federation "Cystic fibrosis"[5].

To conduct the experiment, water-alcohol extracts were obtained from common myrtle leaves by the method of fractional percolation described in the GPhM.1.4.1.0019[6] of the State Pharmacopeia of the Russian Federation XV edition (SPh RF XV ed.), as well as an aqueous infusion of myrtle leaves according to the GPhM.1.4.1.0018[7] SPh RF XV ed.

## Methodology

### Preparation of the working solution

A micromethod was used for the study: testing was performed at a final volume of 100 µl. Working solutions were introduced into micro-dilution plates of 50 µl per well. Using multichannel pipettes, a 96-well sterile tablet for immunological studies (with a flat bottom) with a lid was filled with double serial dilutions of the investigated extracts. The dilutions were then inoculated with a prepared suspension of the investigated microorganism. Incubation was carried out in a normal atmosphere at a temperature of 36°C. During incubation, the tablet was covered with a lid to prevent the contents of the wells from drying out.

### Preparation of inoculum

Inoculum was prepared by suspending colonies selected from a nocturnal culture grown on 5% blood agar (HiMedia, India). The final microbial load in the inoculum was 5 = 105 CFU/mL. To prepare the inoculum with the required concentration of microorganisms, 100 µl of a suspension equivalent to 0.5 according to the McFarland standard was used, which was transferred to a test tube containing 9.9 ml (1:100 dilution) of broth, which made it possible to obtain a suspension with a cell concentration of 1×106 CFU/mL, with the addition of 50 µl of which to an equal volume (50 µl) of the test substance The final composition of the inoculum was obtained from the solution. Inoculum was introduced into test tubes with dilutions of the sample no later than 15 minutes after its preparation. The plates with the tested strains were incubated at 36°C for 24 hours.

### Assessment of microbial growth

The determination of the minimum inhibitory concentration (MIC) and antimicrobial activity was carried out by double serial dilution in broth on test cultures isolated from sputum from patients with cystic fibrosis[8]. To determine the presence of microbial growth, the wells of the crop plates were viewed in transmitted light. The growth of the culture in the presence of the test sample was observed when compared with the hole of the "negative" control. MIC was determined by the lowest concentration of the test sample, which suppresses the visible growth of microorganisms.

### Evaluation of experimental results

The results of microbiological analysis were recorded 48–72 hours after incubation at a temperature of 36°C. From wells with appropriate dilutions of the studied samples with visible growth retardation, seeding was carried out on nutrient media (5% blood agar-agar (HiMedia, India)). After 24 hours, the absence of growth was assessed as a bactericidal effect, and the appearance of visible growth, but with its delay, as bacteriostatic. At the same time, according to the requirements of GOST R ISO 20776-1-2010[9], as well as the recommendations of the Performance Standard for Antimicrobial Sensitivity tests (CLSI)[10], the presence of turbidity and the detection of a small number of microorganisms (1 colony) were not considered when registering the experimental result. The number of repetitions of each experiment was 3.

## RESULTS AND DISCUSSION

As a result of the study, it was found that all water-alcohol extracts from myrtle leaves showed obvious antimicrobial activity, surpassing the control alcohol samples of concentrations 40, 70 and 96 for *Burkholderia cenocepacia, Stenotrophomonas maltophilia* and

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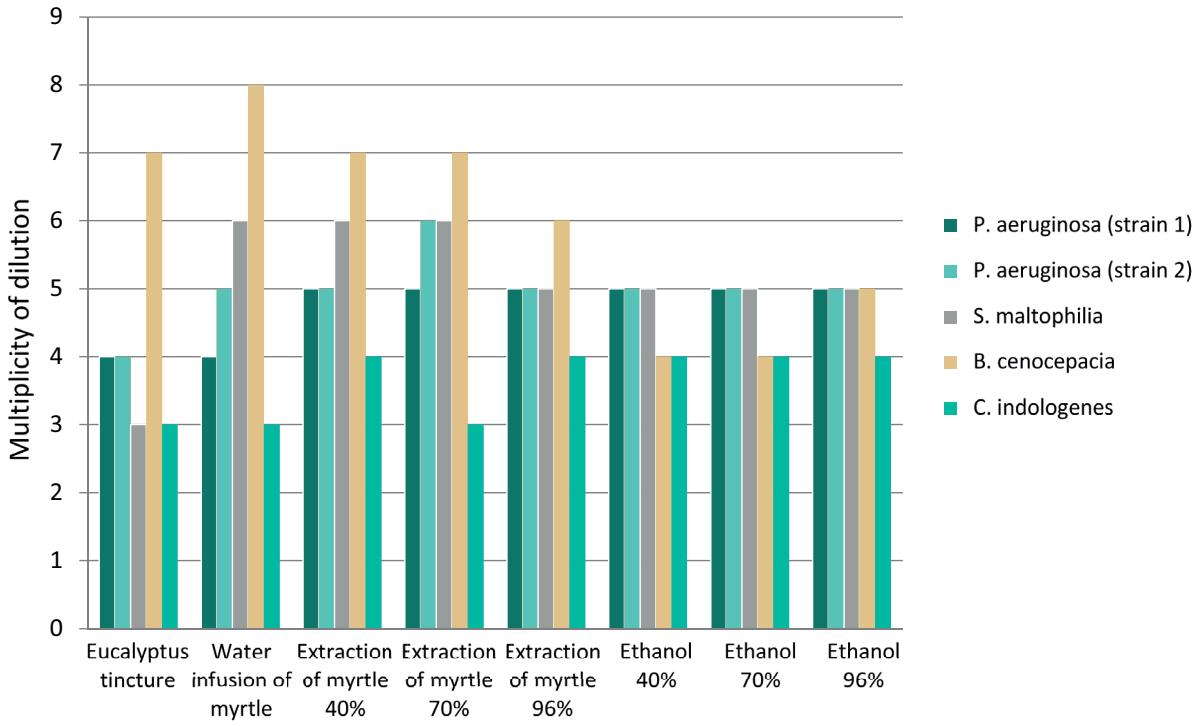


**Figure 1 – Comparative diagram of the antibacterial activity of water-alcohol extracts of common myrtle leaves.**

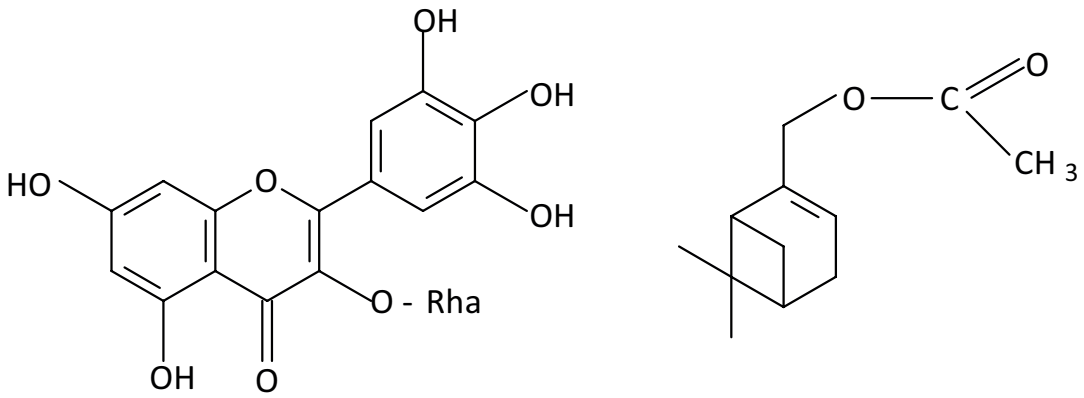


**Figure 2 – Structural formulas of myricitrin (A) and myrtenyl acetate (B).**

*Pseudomonas aeruginosa* strains (mucoid strain 2), and surpassing the comparison sample of eucalyptus tincture for individual strains (Table 1). For two strains (*Pseudomonas aeruginosa* strain 1 and *Chryseobacterium indologenes*), no pronounced antimicrobial activity was detected in the study objects, and the effect was comparable with the control samples (Table 2).

Of all the studied objects, according to the severity of the antimicrobial effect, 2 samples can be distinguished — tincture of myrtle leaves 70% and an aqueous infusion (Fig. 1).

Common myrtle leaf tincture 70% shows the widest bactericidal and bacteriostatic activity among all studied objects in relation to 3 clinical strains of *Burkholderia cenocepacia, Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* (strain 2 mucoid). With respect to the *Burkholderia cenocepacia* strain, the antimicrobial effect of myrtle leaf tincture 70% is comparable to eucalyptus tincture but surpasses it in bactericidal and bacteriostatic activity for two other strains, and also surpasses control samples of ethanol with concentrations of 40, 70 and 96% for all 3 strains by several dilution positions.

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**Table 1 – Results of testing extracts of common myrtle leaves (*M. communis* L.) and comparison preparations**

| Object | Multiplicity of dilution | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
| *Pseudomonas aeruginosa* (strain 1) | | | | | | | | |
| Extraction of myrtle 40% | – | – | – | – | – | + | + | + |
| Extraction of myrtle 70% | – | – | – | – | – | + | + | + |
| Extraction of myrtle 96% | – | – | – | – | – | + | + | + |
| Water infusion of myrtle | – | – | – | – | + | + | + | + |
| Tincture of eucalyptus 70% | – | – | – | – | + | + | + | + |
| *Pseudomonas aeruginosa* (strain 2) | | | | | | | | |
| Extraction of myrtle 40% | – | – | – | – | – | + | + | + |
| Extraction of myrtle 70% | – | – | – | – | – | – | + | + |
| Extraction of myrtle 96% | – | – | – | – | – | + | + | + |
| Water infusion of myrtle | – | – | – | – | – | + | + | + |
| Tincture of eucalyptus 70% | – | – | – | – | + | + | + | + |
| *Stenotrophomonas maltophilia* | | | | | | | | |
| Extraction of myrtle 40% | – | – | – | – | – | – | + | + |
| Extraction of myrtle 70% | – | – | – | – | – | – | + | + |
| Extraction of myrtle 96% | – | – | – | – | – | + | + | + |
| Water infusion of myrtle | – | – | – | – | – | – | + | + |
| Tincture of eucalyptus 70% | – | – | – | + | + | + | + | + |
| *Burkholderia cenocepacia* | | | | | | | | |
| Extraction of myrtle 40% | – | – | – | – | – | – | – | + |
| Extraction of myrtle 70% | – | – | – | – | – | – | – | + |
| Extraction of myrtle 96% | – | – | – | – | – | – | + | + |
| Water infusion of myrtle | – | – | – | – | – | – | – | – |
| Tincture of eucalyptus 70% | – | – | – | – | – | – | – | + |
| *Chryseobacterium indologenes* | | | | | | | | |
| Extraction of myrtle 40% | – | – | – | – | + | + | + | + |
| Extraction of myrtle 70% | – | – | – | + | + | + | + | + |
| Extraction of myrtle 96% | – | – | – | + | + | + | + | + |
| Water infusion of myrtle | – | – | – | + | + | + | + | + |
| Eucalyptus tincture 70% | – | – | – | + | + | + | + | + |

**Table 2 – Minimum suppressive concentrations of ethanol (“negative” control)**

| Object | Multiplicity of dilution* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
| *Pseudomonas aeruginosa* (strain 1) | | | | | | | | |
| Ethanol 40% | – | – | – | – | – | + | + | + |
| Ethanol 70% | – | – | – | – | – | + | + | + |
| Ethanol 96% | – | – | – | – | – | + | + | + |
| *Pseudomonas aeruginosa* (strain 2) | | | | | | | | |
| Ethanol 40% | – | – | – | – | – | + | + | + |
| Ethanol 70% | – | – | – | – | – | + | + | + |
| Ethanol 96% | – | – | – | – | – | + | + | + |
| *Stenotrophomonas maltophilia* | | | | | | | | |
| Ethanol 40% | – | – | – | – | – | + | + | + |
| Ethanol 70% | – | – | – | – | – | + | + | + |
| Ethanol 96% | – | – | – | – | – | + | + | + |
| *Burkholderia cenocepacia* | | | | | | | | |
| Ethanol 40% | – | – | – | – | + | + | + | + |
| Ethanol 70% | – | – | – | – | + | + | + | + |
| Ethanol 96% | – | – | – | – | – | + | + | + |
| *Chryseobacterium indologenes* | | | | | | | | |
| Ethanol 40% | – | – | – | – | + | + | + | + |
| Ethanol 70% | – | – | – | – | + | + | + | + |
| Ethanol 96% | – | – | – | – | + | + | + | + |

The pronounced antimicrobial and antifungal activity of myrtle extracts is due to its component composition, in which the flavonoid myricitrin (3-*O*-*α*-L-rhamnopyranoside of myricetin) and myrtenyl acetate are the dominant BACs[11, 12, 13] (Fig. 2).

It should be noted that 70% ethanol is the optimal extractant for most flavonoid-containing plants, since this concentration of ethanol allows for maximum extraction of the number of flavonoids present in the plant and has a better penetrating ability into the deep layers of the epidermis compared with higher concentrations [18]. In addition, compounds of a terpenoid nature also enter the liquid phase, as we have established during preliminary phytochemical studies. Phenolic substances in the leaves of myrtle are maximally extracted at an ethanol concentration of 70–80% [19].

Foreign clinical and experimental studies show that myrtle leaves, unlike fruits, have a wider range of pharmacological and therapeutic effects, especially such as antibacterial and antifungal [11]. Many foreign studies focus on the antimicrobial activity of water-alcohol extracts from myrtle [20–22]. The antibacterial activity of alcohol extracts of *Myrtus communis* L. was studied by G. Alipour et al. based on 6 gram-positive (*Staphylococcus aureus, Micrococcus luteus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Listeria monocytogenes*) and 4 gram-negative (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Campylobacter jejuni*) bacteria and, according to the results of the study, alcohol extract from myrtle leaves inhibited the growth of all mentioned bacteria, except for the *Campylobacte jejuni* strain. [11]. Moreover, myrtle leaves are a raw source of BACs, which have antimicrobial effects in the treatment of tuberculosis, including against strains of pathogenic bacteria such as *Pseudomonas aeruginosa* [23]. Aquatic extracts of common myrtle leaves (*Myrtus communis* L.) from the southwestern Zagros region in Iran were evaluated. Even though they have antibacterial properties against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa* due to the dominant presence of gallic acid, antifungal activity against *Aspergillus oryzae* has not been observed [24].

The antibacterial activity of alcohol extract of myrtle leaves (*Myrtus communis* L.) was evaluated by MIC, MBC and the size of the inhibition zone against Gram-positive bacteria. Alcoholic myrtle extract demonstrated a significant inhibitory effect against Gram-positive and acid-resistant bacteria, while not affecting the growth of Gram-negative bacteria [25].

The aqueous infusion of myrtle leaves surpassed in bactericidal and bacteriostatic activity the control samples of ethanol with concentrations of 40, 70 and 96% and eucalyptus tincture in relation *to Stenotrophomonas maltophilia* and *Burkholderia cenocepacia* strains. Perhaps this is due to the maximum extraction of gallic acid from an aqueous solution of common myrtle. Also, due to the presence of gallomyrtucommodones in water-alcohol extracts obtained from myrtle leaves, positive results in antimicrobial activity are observed, including against mucoid strains [20].

Thus, water-alcohol extracts from myrtle leaves have antibacterial activity, which may be related to the ability of BACs to inactivate cell membrane transport proteins, enzymes, and microbial adhesion [26]. The mechanisms of antibacterial activity are due to the high content of monoterpene hydrocarbons such as α-pinene, limonene, eucalyptol, linalool, and terpineol, which contribute to the pronounced antimicrobial activity of *M. communis* L. [27, 28]. In addition, an important characteristic of myrtle essential oil and its components is their hydrophobic nature, which allows them to penetrate the lipids of the bacterial cell membrane and disrupt cell function [29]. One of the factors affecting the qualitative composition of the oil, and, accordingly, the degree of severity of the bactericidal and bacteriostatic effects of extracts from MPRM of common myrtle, is its ecological and geographical area of cultivation. It should be borne in mind that there are two main points of view regarding what are the main components of common myrtle essential oil: 1,8-cineol, α-pinene or myrtenyl acetate. Undoubtedly, we can say that both options are correct. While 1,8-cineol and α-pinene predominate in species found in Greece, Italy, France, and Algeria, myrtenyl acetate is found in Portugal, Morocco, Spain, Tunisia, and Albania [30], as well as in Crimea and Krasnodar Territory (Russian Federation).

In the essential oil of myrtle plants of Tunisian and French origin, α-pinene prevails (58.5–52.9%), in Iranian and Italian oils its content is 35–41.6%, which allows it to be attributed to the α-pinene chemotype. The oil obtained in Serbia accumulates the maximum

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amount of linalool (35.7%) and, accordingly, the linalool chemotype can be isolated. The maximum amount of myrtenyl acetate is contained in the oil of winter-hardy myrtle varieties (49.6%) [12]. It can be attributed to the myrtenyl acetate chemotype. The content of 1,8-cineol ranges from 21.6% (Tunisia) to 32.9% (France). These oils, including the one we studied, can be attributed to the 1,8-cineolic chemotype [31]. Thus, the ethereal identity of *Myrtus communis* L. in the conditions of a dry subtropical Mediterranean climate on the southern coast of Crimea, it is 2 times higher than in humid subtropics. An analysis of the biochemical composition of the essential oil indicates a high level of common myrtle myrtenyl acetate (49.6%) and other esters in Crimean myrtle oil, while in humid subtropical conditions significantly more 1,8-cineol is formed [32], which undoubtedly affects the results of studies on antimicrobial activity.

The data obtained during the study indicate that the raw materials of common myrtle leaves are superior in bactericidal and bacteriostatic activity to the medicinal raw materials of the pharmacopoeial plant *Eucalyptus viminalis* L., which provides additional and weighty arguments for the formation of a draft pharmacopoeial article for a new MPRM — Common Myrtle Leaves.

The problem of microbial resistance is growing, and the prospects for the use of antimicrobial drugs in the future are unclear. The vegetable raw materials of myrtle have both pronounced bactericidal and bacteriostatic, antifungal activity, in comparison with ethanol and eucalyptus tincture. Water-alcohol extracts from myrtle based on various concentrations of ethanol (40 and 70%) can be a source of bioflavonoids to develop new medicines with antimicrobial effects based on them. Thus, common myrtle leaves are of interest for further research as a potential MPRM for the treatment of infectious diseases, including in patients with a genetically determined diagnosis of cystic fibrosis.

## CONCLUSION

All the studied samples of water-alcohol extracts from common myrtle leaves exhibit antibacterial activity against strains obtained from patients with cystic fibrosis. It was found that the bactericidal and bacteriostatic activity of common myrtle leaf tincture in 70% alcohol is the most active against three clinical strains of *Burkholderia cenocepacia, Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* (mucoid strain 2), among the studied objects and surpasses the comparison samples in effect. The aqueous infusion of common myrtle leaves surpassed the antimicrobial activity of ethanol samples and eucalyptus tincture in relation to *Stenotrophomonas maltophilia* and *Burkholderia cenocepacia* strains.;

The data obtained during the research allow us to draw conclusions about the future prospects of studying 70% and aqueous infusion of common myrtle leaves for the creation and implementation of medicines based on them in medical and pharmaceutical practice.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTON

Vera D. Maslova — literature analysis, conducting an experiment, data analyzing and interpreting, preparing a draft of the article; Vladimir A. Kurkin — final approval of the article for publication, analysis of the results, critical analysis of the intellectual content; Vitaly M. Ryzhov — development of the concept and design of the study; Artem V. Lyamin — conducting microbiological research, participation in description and analysis of the results; Olga V. Kondratenko — selection of strains of microorganisms, participation in the description and analysis of the results; Nadezhda N. Bakova — collection and identification of raw materials, processing of results, writing of the article; Ekaterina Yu. Bakova — collection and identification of raw materials, preparation of plant material for research, processing of results. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication)

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# Prevalence of *AOX1* and *CYP1A2* gene polymorphisms associated with response to favipiravir therapy in novel coronavirus infection COVID-19 among ethnic groups of the North Caucasus

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Drug sensitivity to, in particular to favipiravir, may vary among representatives of different ethnic groups. Studies have previously shown that carrying certain variants of the *AOX1* and *CYP1A2* genes may be associated with an increased incidence of adverse reactions with patients having COVID-19 and taking favipiravir. This work is devoted to studying the prevalence of mutant variants rs55754655 and rs10931910 of the *AOX1* gene and rs762551 of the *CYP1A2* gene in various ethnic groups of the North Caucasus.

**The aim**. To characterize the distribution structure of *AOX1* (rs55754655 and rs10931910) and *CYP1A2* (rs762551) variants among the peoples of the North Caucasus (Ossetians, Balkars, Kabardians, Avars, Dargins, Laks, Kumyks and Lezgins).

**Materials and methods.** The frequency of distribution of *AOX1* and *CYP1A2* gene variants was studied among 897 conditionally healthy volunteers (362 men — 40.4% and 535 women — 59.6%; average age — 34.6±6.3%), from 8 ethnic groups of the North Caucasus: Ossetians, Balkars, Kabardians, Avars, Dargins, Laks, Kumyks and Lezgins ($n$=100 for each), as well as 97 Russians (reference group).

**Results.** As a result of the analysis, a significant difference was found in the allele frequencies for the rs10931910 *AOX1* genetic polymorphism between Balkars and Russians ($p$ <0.05), Laks and Russians ($p$ <0.05), and especially between Dargins and Russians ($p$ <0.0001). No statistically significant differences in the allele frequencies of the *CYP1A2* gene were found in the comparative analysis of ethnic groups with the comparison group.

**Conclusion**. Significant differences were revealed in the frequency of *AOX1* polymorphisms (rs10931910, rs55754655) in the peoples of the North Caucasus relative to the Russian population. The largest deviations were recorded in Dargins: a decrease in the frequency of the minor allele rs10931910 to 28.5% ($p$ <0.0001) and rs55754655 to 3.0% ($p$=0.0105). The results may be useful for optimizing therapy with medicines that are *AOX1* substrates, which include favipiravir, used to treat patients with COVID-19.

**Keywords**: favipiravir; COVID-19; ethnic groups; *AOX1*; *CYP1A2*

**Abbreviations:** AR — adverse reaction; AOX — aldehyde oxidase; ALT — alanine aminotransferase; AST — aspartate aminotransferase; PCR — polymerase chain reaction; dNTP — deoxynucleotides; TPMT — thiopurine methyltransferase.

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*ОРИГИНАЛЬНАЯ СТАТЬЯ*

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# Распространённость полиморфизмов генов *AOX1* и *CYP1A2*, ассоциированных с ответом на терапию фавипиравиром при новой коронавирусной инфекции COVID-19, среди этнических групп Северного Кавказа

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Чувствительность к лекарственным препаратам, в частности к фавипиравиру, может варьировать у представителей разных этнических групп. В исследованиях ранее показано, что носительство некоторых вариантов генов *AOX1* и *CYP1A2* может ассоциироваться с повышенной частотой нежелательных реакций на фоне приёма фавипиравира у пациентов с COVID-19. Данная работа посвящена изучению распространённости мутантных вариантов rs55754655 и rs10931910 гена *AOX1* и rs762551 гена *CYP1A2* в различных этнических группах Северного Кавказа.

**Цель.** Охарактеризовать структуру распределения вариантов *AOX1* (rs55754655 и rs10931910) и *CYP1A2* (rs762551) среди народов Северного Кавказа (осетин, балкарцев, кабардинцев, аварцев, даргинцев, лакцев, кумыков и лезгинов).

**Материалы и методы.** Изучена частота распределения вариантов генов *AOX1* и *CYP1A2* среди 897 условно здоровых добровольцев (362 мужчин — 40,4% и 535 женщин — 59,6%; средний возраст — 34,6±6,3%), из 8 этнических групп Северного Кавказа: осетины, балкарцы, кабардинцы, аварцы, даргинцы, лакцы, кумыки и лезгины ($n$=100 для каждой), а также 97 русских (группа сравнения).

**Результаты.** В результате анализа было обнаружено достоверное различие в частотах аллелей по генетическому полиморфизму rs10931910 *AOX1* между балкарцами и русскими ($p$ <0,05), лакцами и русскими ($p$ <0,05) и в особенности между даргинцами и русскими ($p$ <0,0001). Статистически значимых различий в частотах аллелей гена *CYP1A2* при сравнительном анализе этногрупп с группой сравнения обнаружено не было.

**Заключение.** Выявлены значимые различия в частоте полиморфизмов *AOX1* (rs10931910, rs55754655) у народов Северного Кавказа относительно русской популяции. Наибольшие отклонения зафиксированы у даргинцев: снижение частоты минорного аллеля rs10931910 до 28,5% ($p$ <0,0001) и rs55754655 до 3,0% ($p$=0,0105). Результаты могут быть полезны для оптимизации терапии препаратами, являющимися субстратами *AOX1*, в число которых входит фавипиравир, применяемый для лечения пациентов с COVID-19.

**Ключевые слова:** фавипиравир; COVID-19; этнические группы; *AOX1*; *CYP1A2*

**Список сокращений:** НР — нежелательная реакция; АОХ — альдегидоксидаза; АЛТ — аланинаминотрансфераза; АСТ — аспартатаминотрансфераза; ПЦР — полимеразная цепная реакция; дНТФ — дезоксинуклеотиды; ТРМТ — тиопуринметилтрансфераза.

## INTRODUCTION

The COVID-19 pandemic has posed a significant challenge to healthcare systems worldwide. The rapid identification and determination of drug treatment options were crucial in combating the outbreak in 2020–2021. It was found out that the genome structure of SARS-CoV-2 was 75–80% identical to the genome sequence of SARS-CoV [1]. This result led to the development of etiotropic therapy regimens using favipiravir, which was indicated for the treatment of SARS and MERS infections. However, the widespread use of favipiravir revealed that this medicine is characterized by the development of a range of adverse reactions (ARs), including elevated liver enzymes (AST / ALT), leukopenia, hyperuricemia, and gastrointestinal disorders [2]. A recent meta-analysis of 25 clinical trials evaluating the use of favipiravir for the treatment of COVID-19 showed that its use is associated with an increased incidence of ARs (OR = 1.27, 95% CI 1.05–1.54; 18 RCTs, 4 699 participants) [2].

Over the past two decades, the field of pharmacogenetics has greatly developed, focusing on the study of the contribution of a patient's genetic profile to the pharmacological effect of the pharmacotherapy they receive. In the context of the development of ARs, the search for possible associations of genetic markers with the safety of the medicine is of interest. Favipiravir is metabolized primarily by aldehyde oxidase 1 (AOX1) to form the inactive metabolite T705M1 and, to a lesser extent, by xanthine oxidase [3]. Possible associations of mutations in the *AOX1* gene with the safety of favipiravir in patients with COVID-19 are currently poorly understood; nevertheless, the use of the "gene-candidate" approach suggests this connection. In addition, *in silico*, using the PASS 2022 program, it was predicted that favipiravir is also a substrate of *CYP1A2* [4].

In our earlier study, which included patients with COVID-19 who received favipiravir in the hospital, we were able to find associations between carrying the rs55754655 and rs10931910 variants of the *AOX1* gene and the rs762551 variant of the *CYP1A2* gene with an increase in the level of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and leukocytes [5]. There is also data on the effect of the rs55754655 polymorphism of the *AOX1* gene on the metabolism of purine compounds, in particular azathioprine [6]. Thus, it can be assumed that in the presence of concomitant diseases requiring the use of medicines that are substrates of *AOX1*, genotyping of the latter will allow timely prescription of adequate therapy and prevention of possible complications. Studies on the dependence between variants of the cytochrome *CYP1A2* gene and the response to drugs metabolized by CYP1A2 are extremely limited. Thus, in a sample of 60 patients, it was shown that the genetic polymorphisms rs2069514 and rs762551 in the *CYP1A2* gene have a statistically significant prognostic value in relation to the severity of COVID-19 [7].

In the works of a number of authors, it was shown that in Russia the frequency of significant pharmacogenetic markers varies between populations and ethnic groups [8–10]. The level of genetic heterogeneity of the populations of Russia, for example, for cytochrome genes, is relatively low, but is closely related to the geographical location of residence of these peoples. Particular attention should be paid to the North Caucasus, which is the most ethnically diverse region and is an ideal object for studying differences in the distribution of genetic variations in ethnic groups [11]. Determining the nature of the carriage of pharmacogenetic markers among local populations remains an important task in the context of the transition to personalized medicine. Knowledge of the structure of the distribution of markers can help identify areas and regions where the introduction of testing will be most priority and justified from the point of view of the healthcare system. This also avoids unnecessary and unjustified prescribing of testing for all patients without the need.

**THE AIM.** In connection with the above information, the aim of this study was to characterize the structure of the distribution of *AOX1* (rs55754655 and rs10931910) and *CYP1A2* (rs762551) variants among the peoples of the North Caucasus (Ossetians, Balkars, Kabardins, Avars, Dargins, Laks, Kumyks and Lezgins) to predict the response to substrates of these enzymes, including favipiravir.

## MATERIALS AND METHODS

### Study design

A cross-sectional retrospective genetic epidemiology study was conducted on conditionally healthy volunteers to determine the population frequency of polymorphisms of the *AOX1* and *CYP1A2* genes, affecting the metabolism and safety of favipiravir according to literature data [3, 5, 12].

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The study involved 897 conditionally healthy volunteers — men (*n* = 362) and women (*n* = 535) from 9 ethnic groups: 100 participants from the Ossetian, Balkar, Kabardin, Avar, Dargin, Lak, Kumyk and Lezgin ethnic groups (North Caucasus) and 97 Russians.

### Eligibility criteria

*Inclusion criteria:* age ≥18 years, ethnicity was determined by self-identification of participants and their parents. There is a high correlation between the self-identification used method and the determination of microsatellite markers of ethnicity as shown in a previous study [13].

*Exclusion criteria*: the study did not include descendants of mixed marriages; refusal to participate in the study.

### Study conditions

The collection of biomaterials was carried out on the bases of the Lak Central District Hospital, the Clinical Hospital of the Ossetian State Medical Academy and the Republican Clinical Hospital.

### Study duration

Biomaterial for analysis was obtained from the bioresource collection of the Russian Medical Academy of Continuing Professional Education, formed in the period from 2015 to 2021. Laboratory genotyping and statistical data processing were carried out in 2024. Thus, the work is a retrospective analysis of the prevalence of alleles in selected samples.

### Genotyping

Genotyping was performed by real-time polymerase chain reaction (PCR). DNA was extracted from 100 µl of venous blood collected in tubes with ethylenediaminetetraacetate (VACUETTE, Greiner Bio-One, Austria). DNA was extracted using "DNA-Extran-1" kits (CJSC "Syntol", Russia) and MagnoPrime UNI (LLC "NextBio", Russia) according to the manufacturer's instructions.

The presence of genetic polymorphisms was detected using reagent kits containing allele-specific TaqMan® probes (Applied Biosystems, USA; TestGen LLC, Russia) on Real-Time CFX96 Touch amplifiers (Bio-Rad Laboratories Inc., USA). PCR was performed in a reaction volume of 10 µl containing genomic DNA — 15 ng, oligonucleotide primers — 0.5 pM, 10X PCR buffer — 1 µl, deoxynucleotides (dNTP) — 250 µM, magnesium chloride — 3 mM and DNA polymerase — 0.25 U. The amplification program included pre-incubation at 95°C for 3 min, then for 50 cycles denaturation at 95°C — 10 s and annealing at 60°C — 30 s. The analysis is based on the detection of a fluorescent signal after each amplification cycle. Genotypes were determined by fluorescence growth curves in the FAM and VIC channels.

### Ethics approval

The study was approved by the Ethics Committee of the Russian Medical Academy of Continuous Professional Education (Protocol No. 15 dated October 16, 2021). At the stage of collection of biomaterials, the study was approved by the Local Ethics Committee of the Republican Clinical Hospital (Protocol No. 2 dated September 05, 2016); of the Lak Central District Hospital (Protocol No. 12 dated September 22, 2015); by the Ethics Committee of the North Ossetian State Medical Academy (Protocol No. 11-3 dated November 16, 2016). The study was conducted in accordance with the legislation of the Russian Federation and International Regulatory Documents (Helsinki Declaration of the World Medical Association, 2013; National Standard of the Russian Federation GOST R 52379-2005).

All participants gave their voluntary informed consent to participate in the study and to the collection and storage of genetic material. According to the terms of informed consent, all study results can be analyzed and published in the relevant scientific journal without disclosing personal information.

### Statistical analysis

The number of samples (100 people per ethnic group) was determined basically on the practice of genetic studies of isolated populations [11, 14].

The Pearson $\chi^2$ criterion ($p < 0.05$) for each gene according to the Hardy-Weinberg law was used to carry out the assessment of the correspondence of the independent distribution of alleles.

The distribution of *AOX1* (rs55754655 and rs10931910) and *CYP1A2* (rs762551) alleles within the Russian ethnic group was used as a reference group.

Russians (*n* = 97), representing the largest ethnic group in Russia, were selected as the reference group. This allows: interpreting the results in the context of All-Russian clinical guidelines; comparing data with previous pharmacogenetic studies [8, 10, 15]; assessing the specificity of the North Caucasian populations relative to the dominant demographic group of the country.

Intergroup comparisons of allele frequencies were performed using the $\chi^2$ criterion (with Yates› correction for small expected frequencies ($n$ = 5–10; $p < 0,05$). The normal distribution was not analyzed, since working with categorical data does not require parametric tests. The results were evaluated in the GraphPad InStat program (GraphPad Software Inc., USA).

## RESULTS

The results of genotyping of subjects by genotype frequency and carriage of variants of the studied markers are presented in tables 1–3.

In most cases, the distribution of *AOX1* and *CYP1A2* genotypes corresponded to the Hardy-Weinberg equilibrium law. Exceptions were *AOX1* rs10931910 T>C ($p$ = 0.0188; Table 1) and *CYP1A2*F1* rs762551 A>C ($p$ = 0.0132; Table 3) in Kabardins, where an excessive number of heterozygous alleles were represented, and *AOX1* rs55754655 T>C in Dargins ($p$ = 0.0018). On the one hand, the Hardy-Weinberg law describes the equilibrium of allele frequencies in a population, but in real conditions there can be disruptive factors. On the other hand, it is necessary to take into account the relatively rare prevalence of the rs55754655 allele in Dargins in comparison with other ethnic groups, statistical errors may occur that require an increase of the number of samples of the Dargin ethnic group for further studies of this genetic polymorphism.

In the context of our study, the results of the analysis revealed significant differences in allele frequencies for rs10931910 of the *AOX1* gene between Balkars and Russians, Dargins and Russians, and Laks and Russians; with respect to rs55754655 of the *AOX1* gene — between Dargins and Russians (Table 4).

No statistically significant differences in the allele frequencies of the *CYP1A2* gene were found in the comparative analysis of ethnic groups.

Despite the relatively small number of samples in each of the comparison groups, there is a noticeable uneven distribution of the allele frequency in the ethnic groups. Thus, the highest occurrence of the minor variant rs10931910 of the *AOX1* gene was 48.5% in Avars and Russians, and the lowest was 28.5% in Dargins. A similar distribution is observed for the rs55754655 alleles of the same gene: the lowest in Dargins (3%), the highest in Ossetians (11.5%), and slightly lower in Russians (9.8%). However, this was not typical for another gene: the highest and lowest percentage of polymorphic allele occurrence was in the Lezgin and Ossetian ethnic groups, 42.5% and 26.5%, respectively.

According to the Ensembl 2024 database [16], the frequency of the C allele rs10931910 of the *AOX1* gene is 43.5%, which is the lowest among all major ethnic groups; for them, this allele is the major one. In our studied samples, the proportion of the minor allele also did not reach 50%. The frequency of the allele variant C rs55754655 of the *AOX1* gene in the European population, on the contrary, is more common than in most others and amounts to 12%. In our samples, the frequencies of this allele are lower than the population average. The minor allele A rs762551 of the *CYP1A2*F1* gene in the large population has a frequency of 32%. The allele frequency of this allele among the ethnic groups of the North Caucasus was higher than 32%, with the exception of Ossetians, Balkars, and Avars.

## DISCUSSION

A comparison of the frequency of occurrence of the studied allele variants in groups was carried out. The peoples of the North Caucasus differ in a more compact area of residence compared to the Russian ethnic group, which lives relatively evenly throughout Russia. The North Caucasus is an excellent example of a region where the distribution of genes can be significantly influenced by the geographical factor; the mountainous isolated nature of settlement determines the genetic structure of the population [15]. Thus, 26 of the 50 autochthonous peoples of the North Caucasus live in the Caspian Sea region. Our study included 5 peoples of the Republic of Dagestan: Avars, Dargins, Laks, Kumyks, and Lezgins. The next ethnic group included in our study is the Ossetians, an Iranian-speaking people living on the northern and southern slopes of the Greater Caucasus Range. Kabardians, a people belonging to the Abkhaz-Adyghe language group, make up the majority of the population of the Kabardino-Balkarian Republic. The second largest ethnic group in the republic is the Balkars, a Turkic-speaking people of the Altai language family. The selection of these populations was due to the criterion that genetic isolation can also be influenced by religious disunity and the absence of a common language (lingua franca) among the Caucasian peoples. Earlier studies have shown that the distribution of pharmacologically significant genetic markers is consistent with this feature of populations living in the North Caucasus; ethnic groups differ significantly with respect to the distribution of pharmacogenetic markers [15, 17].

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**Table 1 – Assessment of the correspondence of *AOX1* rs10931910 T>C genotypes according to the Hardy-Weinberg law**

| Ethnic group (*n*) | Gender (*n*) | Age (M ± SD) | Frequency | Genotype | | | Minor allele frequency, % | Correspondence to Hardy-Weinberg distribution | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TT | TC | CC | | $\chi^2$ | *p* |
| Russians (*n* = 97) | Male (17) Female (80) | 43 ± 12 | observed | 24 | 52 | 21 | 48.45% | 0.5196 | 0.4710 |
| | | | expected | 25.8 | 48.5 | 22.8 | | | |
| | | | % | 24.7% | 53.6% | 21.6% | | | |
| Ossetians (*n* = 100) | Male (27) Female (73) | 33 ± 11 | observed | 35 | 42 | 23 | 44.00% | 2.1823 | 0.1396 |
| | | | expected | 31.4 | 49.3 | 19.4 | | | |
| | | | % | 35.0% | 42.0% | 23.0% | | | |
| Balkars (*n* = 100) | Male (41) Female (49) | 46 ± 19 | observed | 34 | 56 | 10 | 38.00% | 3.5515 | 0.0595 |
| | | | expected | 38.4 | 47.1 | 14.4 | | | |
| | | | % | 34.0% | 56.0% | 10.0% | | | |
| Kabardins (*n* = 100) | Male (38) Female (62) | 47 ± 18 | observed | 25 | 61 | 14 | 44.50% | 5.5198 | **0.0188** |
| | | | expected | 30.8 | 49.4 | 19.8 | | | |
| | | | % | 25.0% | 61.0% | 14.0% | | | |
| Avars (*n* = 100) | Male (79) Female (21) | 24 ± 9 | observed | 27 | 50 | 23 | 48.00% | 0.0003 | 0.9872 |
| | | | expected | 27.0 | 49.9 | 23.0 | | | |
| | | | % | 27.0% | 50.0% | 23.0% | | | |
| Dargins (*n* = 100) | Male (63) Female (37) | 31 ± 15 | observed | 51 | 41 | 8 | 28.50% | 0.0036 | 0.9521 |
| | | | expected | 51.1 | 40.8 | 8.1 | | | |
| | | | % | 51.0% | 41.0% | 8.0% | | | |
| Laks (*n* = 100) | Male (53) Female (47) | 29 ± 9 | observed | 41 | 43 | 17 | 38.12% | 0.9613 | 0.3269 |
| | | | expected | 38.7 | 47.6 | 14.7 | | | |
| | | | % | 40.6% | 42.6% | 16.8% | | | |
| Kumyks (*n* = 100) | Male (20) Female (80) | 34 ± 11 | observed | 37 | 41 | 22 | 42.50% | 2.5961 | 0.1071 |
| | | | expected | 33.1 | 48.9 | 18.1 | | | |
| | | | % | 37.0% | 41.0% | 22.0% | | | |
| Lezgins (*n* = 100) | Male (24) Female (76) | 35 ± 10 | observed | 37 | 53 | 20 | 42.27% | 0.0180 | 0.8933 |
| | | | expected | 36.7 | 53.7 | 19.7 | | | |
| | | | % | 33.6% | 48.2% | 18.2% | | | |

**Table 2 – Assessment of the correspondence of *AOX1* rs55754655 T>C genotypes according to the Hardy-Weinberg law**

| Ethnic group (*n*) | Gender (*n*) | Age (M ± SD) | Frequency | Genotype | | | Minor allele frequency, % | Correspondence to Hardy-Weinberg distribution | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TT | TC | CC | | $\chi^2$ | *p* |
| Russians (*n* = 97) | Male (17) Female (80) | 43 ± 12 | observed | 80 | 15 | 2 | 9.79% | 1.5111 | 0.2190 |
| | | | expected | 78.9 | 17.1 | 0.9 | | | |
| | | | % | 82.5% | 15.5% | 2.1% | | | |
| Ossetians (*n* = 100) | Male (27) Female (73) | 33 ± 11 | observed | 78 | 21 | 1 | 11.50% | 0.1004 | 0.7513 |
| | | | expected | 78.3 | 20.4 | 1.3 | | | |
| | | | % | 78.0% | 21.0% | 1.0% | | | |
| Balkars (*n* = 100) | Male (41) Female (49) | 46 ± 19 | observed | 86 | 14 | 0 | 7.00% | 0.5665 | 0.4516 |
| | | | expected | 86.5 | 13.0 | 0.5 | | | |
| | | | % | 86.0% | 14.0% | 0.0% | | | |
| Kabardins (*n* = 100) | Male (38) Female (62) | 47 ± 18 | observed | 83 | 17 | 0 | 8.50% | 0.8630 | 0.3529 |
| | | | expected | 83.7 | 15.6 | 0.7 | | | |
| | | | % | 83.0% | 17.0% | 0.0% | | | |
| Avars (*n* = 100) | Male (79) Female (21) | 24 ± 9 | observed | 86 | 14 | 0 | 7.00% | 0.5665 | 0.4516 |
| | | | expected | 86.5 | 13.0 | 0.5 | | | |
| | | | % | 86.0% | 14.0% | 0.0% | | | |
| Dargins (*n* = 100) | Male (63) Female (37) | 31 ± 15 | observed | 95 | 4 | 1 | 3.00% | 9.7791 | **0.0018** |
| | | | expected | 94.1 | 5.8 | 0.1 | | | |
| | | | % | 95.0% | 4.0% | 1.0% | | | |
| Laks (*n* = 100) | Male (53) Female (47) | 29 ± 9 | observed | 91 | 9 | 0 | 4.50% | 0.2220 | 0.6375 |
| | | | expected | 91.2 | 8.6 | 0.2 | | | |
| | | | % | 91.0% | 9.0% | 0.0% | | | |
| Kumyks (*n* = 100) | Male (20) Female (80) | 34 ± 11 | observed | 89 | 10 | 1 | 6.00% | 1.2877 | 0.2565 |
| | | | expected | 88.4 | 11.3 | 0.4 | | | |
| | | | % | 89.0% | 10.0% | 1.0% | | | |
| Lezgins (*n* = 100) | Male (24) Female (76) | 35 ± 10 | observed | 88 | 12 | 0 | 6.00% | 0.4074 | 0.5233 |
| | | | expected | 88.4 | 11.3 | 0.4 | | | |
| | | | % | 88.0% | 12.0% | 0.0% | | | |

**Table 3 – Assessment of the correspondence of *CYP1A2*F1* rs762551 A>C genotypes according to the Hardy-Weinberg law**

| Ethnic group (*n*) | Gender (*n*) | Age (M ± SD) | Frequency | Genotype | | | Minor allele frequency, % | Correspondence to Hardy-Weinberg distribution | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | AA | AC | CC | | $\chi^2$ | *p* |
| Russians (*n* = 97) | Male (17) Female (80) | 43 ± 12 | observed | 41 | 44 | 12 | 35.05% | 0.0014 | 0.9707 |
| | | | expected | 40.9 | 44.2 | 11.9 | | | |
| | | | % | 42.3% | 45.4% | 12.4% | | | |
| Ossetians (*n* = 100) | Male (27) Female (73) | 33 ± 11 | observed | 51 | 45 | 4 | 26.50% | 2.4081 | 0.1207 |
| | | | expected | 54.0 | 39.0 | 7.0 | | | |
| | | | % | 51.0% | 45.0% | 4.0% | | | |
| Balkars (*n* = 100) | Male (41) Female (49) | 46 ± 19 | observed | 52 | 39 | 9 | 28.50% | 0.1854 | 0.6667 |
| | | | expected | 51.1 | 40.8 | 8.1 | | | |
| | | | % | 52.0% | 39.0% | 9.0% | | | |
| Kabardins (*n* = 100) | Male (38) Female (62) | 47 ± 18 | observed | 38 | 56 | 6 | 34.00% | 6.1391 | **0.0132** |
| | | | expected | 43.6 | 44.9 | 11.6 | | | |
| | | | % | 38.0% | 56.0% | 6.0% | | | |
| Avars (*n* = 100) | Male (79) Female (21) | 24 ± 9 | observed | 47 | 44 | 9 | 31.00% | 0.0813 | 0.7755 |
| | | | expected | 47.6 | 42.8 | 9.6 | | | |
| | | | % | 47.0% | 44.0% | 9.0% | | | |
| Dargins (*n* = 100) | Male (63) Female (37) | 31 ± 15 | observed | 40 | 44 | 16 | 38.00% | 0.4384 | 0.5079 |
| | | | expected | 38.4 | 47.1 | 14.4 | | | |
| | | | % | 40.0% | 44.0% | 16.0% | | | |
| Laks (*n* = 100) | Male (53) Female (47) | 29 ± 9 | observed | 38 | 42 | 20 | 41.00% | 1.7390 | 0.1873 |
| | | | expected | 34.8 | 48.4 | 16.8 | | | |
| | | | % | 38.0% | 42.0% | 20.0% | | | |
| Kumyks (*n* = 100) | Male (20) Female (80) | 34 ± 11 | observed | 37 | 48 | 15 | 39.00% | 0.0078 | 0.9297 |
| | | | expected | 37.2 | 47.6 | 15.2 | | | |
| | | | % | 37.0% | 48.0% | 15.0% | | | |
| Lezgins (*n* = 100) | Male (24) Female (76) | 35 ± 10 | observed | 35 | 45 | 20 | 42.50% | 0.6286 | 0.4279 |
| | | | expected | 33.1 | 48.9 | 18.1 | | | |
| | | | % | 35.0% | 45.0% | 20.0% | | | |

**Table 4 – Allele frequency of AOX1 (rs55754655 and rs10931910) and CYP1A2 (rs762551) genetic markers among various ethnic groups of the North Caucasus compared to the Russian population**

| Ethnic groups (*n*) | *AOX1* rs10931910 T>C | | | *AOX1* rs55754655 T>C | | | *CYP1A2*F1* rs762551 A>C | | |
|---|---|---|---|---|---|---|---|---|---|
| | Minor allele frequency, *n* (%) | $\chi^2$ | *p* | Minor allele frequency, *n* (%) | $\chi^2$ | *p* | Minor allele frequency, *n* (%) | $\chi^2$ | *p* |
| Russians (*n* = 97) | 94 (48.5%) | – | – | 19 (9.8%) | – | – | 68 (35.1%) | – | – |
| Ossetians (*n* = 100) | 88 (44.0%) | 0.786 | 0.3754 | 23 (11.5%) | 0.301 | 0.5832 | 53 (26.5%) | 3.384 | 0.0658 |
| Balkars (*n* = 100) | 76 (38.0%) | 4.387 | **0.0362** | 14 (7.0%) | 1.002 | 0.3169 | 57 (28.5%) | 1.951 | 0.1624 |
| Kabardins (*n* = 100) | 89 (44.5%) | 0.619 | 0.4315 | 17 (8.5%) | 0.199 | 0.6559 | 68 (34%) | 0.048 | 0.8263 |
| Avars (*n* = 100) | 96 (48.0%) | 0.008 | 0.9282 | 14 (7.0%) | 1.002 | 0.3169 | 62 (31.0%) | 0.731 | 0.3925 |
| Dargins (*n* = 100) | 57 (28.5%) | 16.588 | **<0.0001** | 6 (3.0%) | 6.548 | **0.0105** | 76 (38.0%) | 0.369 | 0.5435 |
| Laks (*n* = 100) | 77 (38.5%) | 3.972 | **0.0463** | 9 (4.5%) | 3.417 | 0.0645 | 82 (41.0%) | 1.478 | 0.2241 |
| Kumyks (*n* = 100) | 85 (42.5%) | 1.408 | 0.2354 | 12 (6.0%) | 1.955 | 0.162 | 78 (38.0%) | 0.658 | 0.4172 |
| Lezgins (*n* = 100) | 83 (41.5%) | 1.924 | 0.1654 | 12 (6.0%) | 1.955 | 0.162 | 85 (42.5%) | 2.3 | 0.1294 |

Information of the distribution of clinically significant markers among ethnic groups of the population makes it possible to identify regions with increased sensitivity to certain drugs. From a practical point of view, the study of the carriage of polymorphic alleles of genes involved in the biotransformation and effects of drugs in various populations is relevant from the perspective of optimizing pharmacogenetic studies and introducing such testing into routine practice in individual regions.

Cytochrome P450 (CYP) is a superfamily of monooxygenases containing heme as a cofactor and found in all cells and tissues of mammals, with the exception of mature erythrocytes and skeletal muscle tissue cells [18]. CYPs are most studied as enzymes that metabolize drugs. For *CYP1A2*, the substrate of

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which, according to in silico modeling, is favipiravir, some relationships have been identified between variants of the enzyme gene and changes in the drug response to clozapine, paroxetine, opioids, and escitalopram [19]. The frequency of ARs to drugs is higher in carriers of alleles that reduce the activity of the enzyme, which leads to a decrease in metabolism and its elimination from the body, which causes the manifestation of toxic effects. In the context of our study, we did not find that the frequency of the rs762551 allele of the *CYP1A2* gene differs in the studied groups. Thus, it can be assumed that the population of the North Caucasus region does not stand out for increased sensitivity to favipiravir. However, further study of the contribution of *CYP1A2* and its variants to changes in the metabolism and effects of the medicine is necessary.

Aldehyde oxidase (AOX) is a molybdenum-containing flavoenzyme involved in phase I of medicine metabolism [20]. Four isoforms of AOX have been identified in mammals, but only *AOX1* is the functional gene among them [21]. The *AOX1* protein is localized in the cytoplasm and is mainly represented as monomers and homodimers. Its function is to catalyze the oxidation of many different aldehydes and heterocyclic medicine molecules containing nitrogen atoms, such as azathioprine, famciclovir, and methotrexate [22, 23], as well as to catalyze the reduction of nitrogenous aromatic compounds, such as nitrazepam and dantrolene [12, 24]. The results of several studies indicate the clinical significance of interactions between medicine molecules that are substrates of *AOX1*, for example, between methotrexate and favipiravir in a patient with osteosarcoma [25]. Given this, inhibition of *AOX1* may be an effective approach that blocks the metabolism of methotrexate and thereby increases its effectiveness [26]. Thus, it is necessary to take into account the enzymatic activity of *AOX1* when, with the use of standard doses of medicines that are substrates of *AOX1*, the patient does not observe an adequate response to therapy. In our study, on a sample including 100 volunteers from various ethnic groups of the North Caucasus, data were obtained on the heterogeneity of the distribution of polymorphisms rs10931910 and rs55754655 of the *AOX1* gene. The greatest differences from the Russian population among the peoples of the North Caucasus were demonstrated by variants of the *AOX1* gene. Thus, in addition to the lower prevalence of the C allele in the rs55754655 polymorphism in Dargins (3% compared to 9.8% in Russians, $p = 0.0105$), there is also a decrease in the frequency of the risk allele C in rs10931910 T>C compared to the Russian population (48.5%), Balkars (38%), Laks (38.5%) and Dargins (28.5%; $p < 0.0001$). These observations probably indicate the degeneration of risk alleles in the *AOX1* gene in certain populations of the North Caucasus.

To date, there are data from isolated studies that have shown an association between individual polymorphisms of the *AOX1* gene and the response to therapy with azathioprine [27], allopurinol [28], and the antitumor medicine XK469 [29]. Thus, in the presence of the rs55754655 T>C mutation in combination with an increased level of thiopurine methyltransferase (TPMT), only 33% of patients demonstrated a normal response to azathioprine therapy. Conversely, when both of these factors were favorable (T allele in rs55754655 in combination with a normal TPMT level), a normal response to azathioprine therapy was observed in 86% of cases [27]. Genetic polymorphisms rs11678615 C>T, rs3731722 A>G, and rs75995567 T>C, in turn, led to the need to increase the dose of allopurinol to 300 mg/day or more [28]. Finally, the rs10931910 T>C mutation led to a 41% slowdown in the elimination of the antitumor drug XK469 from the body in the case of the TC heterozygote and 67% in the case of the CC homozygote during therapy for solid tumors [29].

The official instructions for the medical use of favipiravir[1], available on the website of the Russia State Registry of Medicines, indicate the medicine interaction of favipiravir with pyrazinamide, repaglinide, theophylline, famciclovir, sulindac, and it is the interaction with the latter two drugs that is associated with the inhibition of AOX by favipiravir.

Thus, further study of the clinical significance of *AOX1* gene polymorphisms is necessary when prescribing medicines whose biotransformation is associated with the *AOX1* enzyme, as well as medicine interactions at the *AOX1* level, including favipiravir, which is especially important for patients with concomitant diseases in which other *AOX1* substrate drugs may be prescribed. Genotyping of the polymorphisms we studied may in the future become one of the important ways to improve the effectiveness and safety of therapy for patients with COVID-19.

### Limitations of the study

The main study limitations are the lack of a preliminary sample size calculation. Although the size of the groups ($n = 100$ for the ethnic groups of the Caucasus, $n = 97$ for Russians) corresponds to the standards of population studies [11, 13, 15] and provides acceptable accuracy for allele frequencies >5% (error ≤7%), for rare variants (for example, rs55754655 with a frequency of 3% in Dargins), the estimation error reaches 3.4% (95% CI: 0.6–8.5%), which may affect the reliability of the identified intergroup differences.

## CONCLUSION

As a result of the study, the population frequency of clinically significant polymorphisms of the *AOX1* (rs55754655, rs10931910) and *CYP1A2* (rs762551)

[1] Favipiravir. Russia State Registry of Medicines. Available from: https://grls.rosminzdrav.ru/GRLS.px?RegNumber=&MnnR=фавипиравир&lf=&TradeNmR=&OwnerName=&MnfOrg=&MnfOrgCountry=&isfs=0&regtype=1%2c6&pageSize=10&token=aa088037-2cb7-4cb6-94aa-b1f-60b5e5ba5&order=Registered&orderType=desc&pageNum=1

genes in ethnic groups of the North Caucasus was characterized. Statistically significant differences were established in the distribution of *AOX1* alleles between the Russian reference group and the indigenous peoples of the region: lower frequencies of the minor allele rs10931910 in Balkars (38.0 *vs* 48.5%, $p = 0.036$), Laks (38.5 *vs* 48.5%, $p = 0.046$) and Dargins (28.5 *vs* 48.5%, $p < 0.0001$); rare occurrence of the risk allele rs55754655 was noted in Dargins (3.0 *vs* 9.8%, $p = 0.0105$).

The presented data create the basis for personalized prescription of *AOX1* substrates, including favipiravir, in the North Caucasus region. Carriage of the identified variants is associated with a change in medicine metabolism and the risk of ARs (hepatotoxicity, leukopenia). At the same time, the clinical significance of these polymorphisms requires further verification in prospective studies involving patients receiving therapy.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

Arvo T. Leinsoo — writing the text of the article, performing the laboratory part of the research; Natalia P. Denisenko, Sherzod P. Abdullaev — critical evaluation of the results and the text of the article; Svetlana N. Tuchkova — statistical analysis of the results; Alexander V. Kryukov, Suleiman N. Mammaev, Zhannet A. Sozaeva, Mariam S.-Kh. Sozaeva, Kristina A. Akmalova, Laura Z. Bolieva — selection of study participants, collection of biological material; Alina I. Dobroselskaya — performing the laboratory part of the research; Maxim L. Maximov, Karin B. Mirzaev, Dmitry A. Sychev — scientific consulting. All the authors have made an equivalent and equivalent contribution to the preparation of the publication. All authors confirm their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the research, and preparation of the article, read and approved the final version before publication).

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# Original drugs approved by the Food and Drug Administration (Center for Drug Evaluation and Research) in 2024

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The U.S. Food and Drug Administration (FDA), in particular the Center for Drug Evaluation and Research (CDER), plays a key role in ensuring the safety, efficacy, and innovation of medicines entering the U.S. market, and then the world. The annual review of new medicines approved by the FDA is an important tool for analyzing current trends in pharmacology and medicine, reflecting progress in the treatment of complex diseases, including cancers, orphan diseases, and infections. The review is compiled to familiarize medical specialists and pharmacologists with current trends in the registration of original medicines and in the therapy of malignant neoplasms, orphan diseases.

**The aim.** To summarize and systematize data on the newest medicines that entered the market in 2024, as well as to analyze the mechanisms of their action. The article aims to inform medical specialists and pharmacologists about current trends in the development and registration of innovative medicines in 2024.

**Materials and methods.** The presented data are taken from open sources and supplemented with the results of individual studies on new mechanisms and approaches in therapy. The main list of new drugs and introductory information about them are taken from the FDA report "Novel Drug Approvals for 2024". Data on medicine prescriptions, as well as information on the mechanism of action, are taken from published summary of product characteristics (SmPC) published on this resource, as well as from the Drugs.com website. To describe previously registered medicines for which a new indication is presented, Drugs.com reports were also used. Structural formulas of drugs are taken from the PubChem resource. In case of the absence of structural formula, data from their SmPC or third-party resources, such as Drugbank, were used. The search for literature data on fundamental studies relating to the mechanisms of action of the presented medicines was carried out in the PubMed, ResearchGate, Google Scholar and elibrary.ru databases.

**Results.** A statistical analysis of registrations, the dynamics of changes in the shares of various types of medicines and basic data on new original drugs registered by CDER are presented. In 2024, the FDA registered 50 original medicines, among which 48% contain a "first-in-class" molecule as an active substance. Small molecules include active substances — 60%, and biopharmaceuticals — 34% (the remaining 6% are imaging agents). At the same time, monoclonal antibodies (mAb) of antitumor and anti-inflammatory action occupy a larger share among biopharmaceuticals.

**Conclusion.** The large proportion of biopharmaceuticals among those newly registered in 2024 emphasizes the dynamic development of the pharmaceutical industry and its focus on personalized medicine and biotechnology. Therapy based on mAbs interacting with receptors, as well as immunotherapy based on newly discovered mechanisms of antitumor immunity, occupies a separate part in the structure of registered original medicines. The search for new rational combinations of

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antibiotics remains relevant. Most of the original drug market is still made up of small molecules, among which there are medicines — ligands of new targets and oligonucleotide sequences.

**Keywords:** FDA; original drugs; immunotherapy; small molecules; biopharmaceuticals; medicines for orphan diseases treatment

**Abbreviations:** BCG — Bacillus Calmette-Guerin; MIC — minimum inhibitory concentration; NSCLC — non-small cell lung cancer; SmPC — summary of product characteristics; PTH — parathyroid hormone; UDCA — ursodeoxycholic acid; cAMP — cyclic adenosine monophosphate; cGMP — cyclic guanosine monophosphate; ADCC — antibody-dependent cell-mediated cytotoxicity; ALK — anaplastic lymphoma kinase; CD — cluster of differentiation; CDER — Center for Drug Evaluation and Research; CFTR — cystic fibrosis transmembrane regulator; CLDN18.2 — claudin 18.2; CRF — corticotropin-releasing factor; CXCR4 — chemokine receptor that regulates cell migration in the immune system; EGF — epidermal growth factor; EGFR — epidermal growth factor receptor; ESBL — extended-spectrum beta-lactamase; Fc-fragment — crystallizing fragment of immunoglobulin; FcR — receptor for the Fc-fragment; FDA — US Food and Drug Administration; HER — human epidermal growth factor receptor; HR — hormone receptor; IFN — interferon; Ig — immunoglobulin; mAb — monoclonal antibody; MRSA — methicillin-resistant *Staphylococcus aureus*; MSSA — methicillin-sensitive *Staphylococcus aureus*; NK — natural killer; NPC — mutation causing Niemann-Pick disease type C; OAT3— organic anion transporter 3; PBP — penicillin-binding protein; PD — programmed cell death receptor, or death receptors; PD-L — programmed cell death receptor ligand; PH — hypoxia-inducible prolyl hydroxylase; PPAR — peroxisome proliferator-activated receptors; SDF-1α/CXCL12 — stromal cell factor 1α / ligand 12 to chemokine CXC; TFPI — tissue factor pathway inhibitor; TGF — transforming growth factor; TLR — Toll-like receptor; TNF — tumor necrosis factor; VEGF — vascular endothelial growth factor; VEGFR — vascular endothelial growth factor receptor.

# Оригинальные лекарственные препараты, одобренные Food and Drug Administration (Center for Drug Evaluation and Research) в 2024 году

**Д.В. Куркин[1, 2], Н.А. Осадченко[1], А.Р. Макарова[1], Д.А. Галкина[1], Д.А. Бакулин[1], О.В. Шаталова[2], А.В. Стрыгин[2], В.И. Петров[2], О.В. Маринчева[1], Ю.В. Горбунова[1], Ю.А. Колосов[1], А.В. Заборовский[1], Д.В. Юнина[1], К.Н. Корянова[3], Е.И. Морковин[1], М.А. Джавахян[1], В.И. Зверева[1], Р.В. Драй[4], И.Е. Макаренко[4], А.С. Шуваева[1, 5]**

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Управление по санитарному надзору за качеством пищевых продуктов и медикаментов США (U.S. Food and Drug Administration, FDA), в частности Центр по оценке и изучению лекарственных препаратов (Center for Drug Evaluation and Research, CDER), играет ключевую роль в обеспечении безопасности, эффективности и инновационности лекарственных препаратов (ЛП), поступающих на рынок США, а затем и всего мира. Ежегодный обзор новых ЛП, одобренных FDA, представляет собой важный инструмент для анализа современных тенденций в фармакологии и медицине, отражая прогресс в лечении сложных заболеваний, включая онкологические патологии, орфанные болезни и инфекционные процессы. Обзор составлен с целью ознакомления медицинских специалистов и фармакологов с современными тенденциями в регистрации оригинальных ЛП и в терапии злокачественных образований, орфанных болезней.

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**Цель.** Обобщение и систематизация данных о новейших ЛП, вышедших на рынок в 2024 году, а также анализ механизмов их действия. Статья направлена на информирование медицинских специалистов и фармакологов в части современных тенденций в разработке и регистрации инновационных ЛП в 2024 году.

**Материалы и методы.** Представленные данные взяты из открытых источников и дополнены результатами отдельных исследований, посвящённых изучению новых механизмов и подходов в терапии. Основной список новых ЛП и вводная информация о них взяты из отчета FDA «Novel Drug Approvals for 2024». Данные по назначениям ЛП, а также информация о механизме действия, взяты из опубликованных общих характеристик лекарственных препаратов (ОХЛП), опубликованных на этом ресурсе, а также с сайта Drugs.com. Для описания ранее зарегистрированных лекарственных препаратов, для которых представлено новое назначение, также использованы отчеты Drugs.com. Структурные формулы ЛП взяты с ресурса PubChem. В случае отсутствия структурной формулы на этом ресурсе использовали данные их ОХЛП, либо сторонние ресурсы, например Drugbank. Поиск литературных данных о фундаментальных исследованиях, касающихся механизмов действия представленных ЛП осуществляли в базах данных PubMed, ResearchGate, Google Академия и elibrary.ru.

**Результаты.** Приведён статистический анализ регистраций, динамика изменения долей различных видов ЛП и основные данные о новых оригинальных ЛП, зарегистрированных CDER. За 2024 год в FDA было зарегистрировано 50 оригинальных ЛП, среди которых 48% ЛП в качестве активного вещества содержат «первую в классе» молекулу. К малым молекулам относятся активные субстанции — 60% ЛП, а к биопрепаратам — 34% (оставшиеся 6% — визуализирующие агенты). При этом среди биопрепаратов бóльшую долю занимают моноклональные антитела (mAb) противоопухолевого и противовоспалительного действия.

**Заключение.** Большая доля биопрепаратов среди вновь зарегистрированных ЛП в 2024 году подчёркивает динамичное развитие фармацевтической отрасли и ее ориентацию на персонализированную медицину и биотехнологии. Терапия, основанная на mAb, взаимодействующих с рецепторами, а также иммунотерапия, основанная на новых открытых механизмах противоопухолевого иммунитета, занимает отдельную часть в структуре зарегистрированных оригинальных ЛП. Остаётся актуальным поиск новых рациональных комбинаций антибиотиков. Большую часть рынка оригинальных ЛП все еще составляют малые молекулы, среди которых появляются ЛП — лиганды новых мишеней и олигонуклеотидные последовательности.

**Ключевые слова:** FDA; оригинальные препараты; иммунотерапия; малые молекулы; биопрепараты; препараты для лечения орфанных болезней

**Список сокращений:** БЦЖ — бацилла Кальметта-Герена; МПК — минимальная подавляющая концентрация; НМРЛ — немелкоклеточный рак легкого; ОХЛП — общая характеристика лекарственного препарата; ПТГ — паратиреоидный гормон; УДХК — урсодезоксихолевая кислота; цАМФ — циклический аденозинмонофосфат; цГМФ — циклический гуанозинмонофосфат; ADCC — антиген-зависимая клеточная цитотоксичность; ALK — киназа анапластической лимфомы; CD — кластер дифференцировки; CDER — Центр по оценке и изучению лекарственных препаратов; CFTR — трансмембранный регулятор муковисцидоза; CLDN18.2 — клаудин 18.2; CRF — кортикотропин-релизинг фактор; CXCR4 — хемокиновый рецептор, который регулирует миграцию клеток в иммунной системе; EGF — эпидермальный фактор роста; EGFR — рецептор эпидермального фактора роста; ESBL — бета-лактамаза расширенного спектра; Fc-фрагмент — кристаллизующийся фрагмент иммуноглобулина; FcR — рецептор к Fc-фрагменту; FDA — Управление по санитарному надзору за качеством пищевых продуктов и медикаментов США; HER — рецептор эпидермального фактора роста человека; HR — рецептор гормона; IFN — интерферон; Ig — иммуноглобулин; mAb — моноклональное антитело; MRSA — устойчивые к метициллину *Staphylococcus aureus*; MSSA — чувствительные к метициллину *Staphylococcus aureus*; NK — натуральный киллер; NPC — мутация, вызывающая болезнь Ниманна-Пика типа С; OAT3— переносчик органических анионов 3; PBP — пенициллинсвязывающий белок; PD — рецептор запрограммированной клеточной гибели, или рецепторы смерти; PD-L — лиганд рецептора запрограммированной клеточной гибели; PH — индуцируемая гипоксией пропилгидроксилаза; PPAR — рецепторы, активируемые пролифераторами пероксисом; SDF-1α/CXCL12 — стромальный клеточный фактор 1α / лиганд 12 к химокину CXC; TFPI — ингибитор пути тканевого фактора; TGF — трансформирующий фактор роста; TLR — Toll-подобный рецептор; TNF — фактор некроза опухоли; VEGF — фактор роста эндотелия сосудов; VEGFR —рецептор фактора роста эндотелия сосудов.

## INTRODUCTION

Increasing life expectancy, improving its quality, and preserving and restoring health are priority areas for medical and social services, with a multidisciplinary approach appearing to be the only possible one to solving these problems [1]. Pharmacotherapy is the main element of human health management, and life expectancy and its quality directly depend on the availability of innovative medicines [2]. Modern pharmacy is one of the most science, technology-, and resource-intensive industries and occupies a leading position in attracting investment [3, 4]. The global pharmaceutical market is constantly undergoing processes, tending to help the largest companies dominate through the creation of advantages, including the development and implementation of various kinds of innovations[1]. Medicines can traditionally be divided into several types — original (innovative), a new dosage form or delivery system of a previously known drugs, combined, reproduced, or those registered for new indications. It is important to take into account the existence of non-equivalent exchange in resources and their unequal

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availability (financial, labor, technological, logistical, and many others, the use of which is necessary throughout the life cycle of a medicine from idea to post-marketing monitoring). The creation of an original medicine is traditionally considered an extremely science-intensive, lengthy, and risky process, while the development of a generic or medicine in a new dosage form requires a developed technological infrastructure and an effectively built marketing component [5]. However, though the development of developing biosimilar medicines is similar to the process of creating a generic, specialists have to re-develop the original product[2], using reverse engineering methods. Registration of a drug for new indications requires reliable evidence of efficacy and safety, which is impossible without a perfectly built system for organizing and conducting clinical trials (CTs): the development of reproduced drugs will be unprofitable if there is no developed marketing system for implementation and promotion[3, 4]. The above facts reflect the increasing (as science, technology, and competition develop) complexity and dynamism of the processes taking place in the field of drug development and research, while integration into the global market multiplies the requirements for applicants[5].

The success of domestic pharmaceutical companies in 2024 indicates abilities and impressive results in the development of both original and reproduced medicines. Thus, the company JSC "R-Pharm" (Russia) registered a drug with the trade name Artserix® (INN: goflicept) for the treatment of such an orphan disease as idiopathic recurrent pericarditis (indications for use may be expanded during clinical trials) and a drug with the trade name Viltepso® (INN: viltolarsen) for the treatment of Duchenne muscular dystrophy with a confirmed mutation in the dystrophin protein gene, amenable to exon 53 skipping. Also, the company JSC "R-Pharm" received the right to conduct clinical trials of generics of anticancer drugs with the trade name Zenlistik® (INN: abemaciclib) and Lynparza® (INN: olaparib), and a drug for the treatment of hepatitis C — Maviret® (glecaprevir+pibrentasvir); biosimilar of the drug Keytruda® with the trade name Arfleida® (INN: pembrolizumab).

The company JSC "Generium" registered a drug with the trade name Lantesens® (nusinersen, analogue of Spinraza®) for the treatment of spinal muscular atrophy. The company PJSC "Promomed" registered an drug with the trade name Velgia® (INN: semaglutide; also received permission to conduct phase I CTs of a generic drug with INN tirzepatide from Eli Lilly), LLC "Geropharm" — Semavik® (in 2024 they also registered RinGluzin® [INN: insulin glulisine] and the company received the right to conduct CT of its own long-acting insulin — GP40201), and the company LLC "PSK Pharma" — Insudaiv® (also registered "Tedizolid PSK®" in 2024 [INN: tedizolid]), which are generics of the original Ozempic® (Novo Nordisk). The company LLC "Petrovax Pharm" registered a medicine with the trade name Areima® (INN: camrelizumab) — an anticancer drug used in the treatment of esophageal and nasopharyngeal cancer. The company "Biocad" was granted permission to conduct phase III CT of the first Russian gene therapy drug in the form of a solution for infusions for the treatment of hemophilia type B.

In the field of academic science and development, several important facts can be noted. For example, the St. Petersburg State Chemical Pharmaceutical University attracts investors to conduct Phase II clinical trials of three of its own drugs developed on the basis of the synthesis of original molecules. The National Medical Research Center for Hematology received permission from the Ministry of Health of the Russian Federation to conduct Phase I–II CTs of the first Russian cell gene therapy (CAR-T) drug, which received the trade name Utzhefra® (INN: hemagenlecleucel). The Siberian State Medical University announced the completion of Phase I of CTs of two original drugs (a cholesterol-lowering agent and an antitumor agent), as well as the early stage of development of an innovative medicine that increases bone tissue regeneration (potentially in demand in dentistry and cosmetology). Three Russian institutions announced the development of vaccines against HIV infection.

In 2024, the U.S. Food and Drug Administration (FDA) confirmed the registration of 50 medicines that are classified as "original" (Table 1).

**THE AIM.** To systematize and analyze current trends in the development of new medicines registered with the FDA in 2024, with a focus on innovative mechanisms of action and their application in oncology, treatment of rare (orphan) diseases, and infections. This review aims to inform medical professionals and pharmacologists about current trends in the development and registration of innovative medicines in 2024.

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**Table 1 – Medicines registered with the U.S. Food and Drug Administration in 2024**

| Registration date | Trade name | Manufacturer | INN | Pharmaceutical form | Class | Indication |
|---|---|---|---|---|---|---|
| Dec 20 | Alhemo | Novo Nordisk Inc. | Concizumab-mtci | Solution for subcutaneous administration | Monoclonal antibody | Reducing the frequency of bleeding episodes in adults and children over 12 years of age with hemophilia A and hemophilia B |
| Dec 20 | Alyftrek | Vertex Pharmaceuticals Incorporated | Vanzacaftor+tezacaftor +deutivacaftor | Tablets for oral administration | Regulatory protein ligand | Cystic fibrosis |
| Dec 19 | Tryngolza | Ionis Pharmaceuticals, Inc. | Olezarsen | Solution for subcutaneous administration | Oligonucleotide | Familial chylomicronemia |
| Dec 18 | Ensacove | Xcovery Holdings, Inc | Ensartinib | Capsules for oral administration | Kinase inhibitor | NSCLC |
| Dec 13. | Crenessity | Neurocrine Biosciences, Inc. | Crinecerfont | Capsules for oral administration or solution for oral administration | Selective CRH 1 antagonist | An adjunct to glucocorticoid replacement therapy for the control of androgens in adults and children aged 4 years and older with classic congenital adrenal hyperplasia |
| Dec 13 | Unloxcyt | Checkpoint Therapeutics, Inc. | Cosibelimab-ipdl | Solution for intravenous administration | Antibody | Metastatic or locally advanced cutaneous squamous cell carcinoma when radiotherapy or surgery is not possible |
| Dec 04 | Bizengri | Merus N.V | Zenocutuzumab-zbco | Solution for intravenous administration | Bispecific antibody to HER2 and HER3 | NSCLC |
| Nov 27 | Iomervu | BIPSO GmbH | Iomeprol | Solution for intra-arterial or intravenous administration | Radiographic contrast | Visualization during intra-arterial and intravenous procedures |
| Nov 22 | Rapiblyk | AOP Orphan Pharmaceuticals GmbH | Landiolol | Solution for intravenous administration | Beta-blocker | Short-term reduction of ventricular rate in adult patients with supraventricular tachycardia, including atrial fibrillation or flutter |
| Nov 22 | Attruby | BridgeBio Pharma, Inc. | Acoramidis | Tablets for oral administration | Transthyretin quaternary structure stabilizer | Transthyretin amyloid cardiomyopathy |
| Nov 20 | Ziihera | Jazz Pharmaceuticals Ireland Limited | Zanidatamab-hrii | Solution for intravenous administration | HER2 antibody | Previously treated, unresectable or metastatic bile duct cancer positive for HER2 mutation (IHC 3+) |

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| Registration date | Trade name | Manufacturer | INN | Pharmaceutical form | Class | Indication |
|---|---|---|---|---|---|---|
| Nov 15 | Revuforj | Syndax Pharmaceuticals | Revumenib | Tablets for oral administration | Menin inhibitor | Relapsed or refractory acute leukemia with lysine methyltransferase 2A (KMT2A) gene translocation in children from 1 year and adults |
| Oct 25 | Orlynvah | Iterum Therapeutics U.S. Limited | Sulopenem etzadroxil and probenecid | Tablets for oral administration | Carbapenem + inhibitor of transport through renal tubules | Urinary tract infections caused by *Escherichia coli, Klebsiella pneumoniae* or *Proteus mirabilis* |
| Oct 18 | Vyloy | Astellas Pharma US, Inc. | Zolbetuximab-clzb | Solution for intravenous administration | Antibody against claudin 18.2 | Combined with fluoropyrimidine or platinum-based therapy for patients with locally advanced unresectable or metastatic HER2-negative CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma |
| Oct 11 | Hympavzi | Pfizer Inc. (Pfizer Labs) | Marstacimab-hncq | Solution for subcutaneous administration | Antagonist of the tissue factor pathway inhibitor | Reduction in the frequency of bleeding episodes in adults and children over 12 years of age with hemophilia A and hemophilia B |
| Oct 10 | Itovebi | Genentech USA, Inc. | Inavolisib | Tablets for oral administration | Kinase inhibitor | Locally advanced or metastatic breast cancer, provided it is endocrine-resistant, has a PIK3CA mutation, HR-positive, HER2-negative after relapse, during or after completion of adjuvant endocrine therapy |
| Sep 27 | Flycardo | GE Healthcare Inc. | Flurpiridaz | Solution for intravenous administration | Radiopharmaceutical for positron emission tomography | Myocardial perfusion imaging with positron emission tomography |
| Sep 26 | Cobenfy | Bristol-Myers Squibb Company | Xanomeline and trospium chloride | Capsules for oral administration | Muscarinic receptor agonist + antagonist | Schizophrenia in adults |
| Sep 24 | Aqneursa | IntraBio Inc. | Levacetylleucine | Suspension for oral administration | Amino acid derivation | Niemann-Pick disease type C in children with body weight >15 kg and in adults |
| Sep 20 | Miplyffa | Zevra Therapeutics, Inc. | Arimoclomol | Capsules for oral administration | Drug for the treatment of ALS with an unknown mechanism of action | Niemann-Pick disease type C in children over 2 years of age and in adults |
| Sep 13 | Ebglyss | Eli Lilly and Company | Lebrikizumab-lbkz | Solution for subcutaneous administration | Interleukin 13 antagonist | Moderate to severe atopic dermatitis in children over 12 years of age and adults, with a body weight of at least 40 kg, with ineffectiveness or contraindications to the use of topical drugss |
| Aug 19 | Lazcluze | Janssen Biotech, Inc | Lazertinib | Tablets for oral administration | Kinase inhibitor | NSCLC with exon 19 deletion or L858R substitution in exon 21 of the *EGFR* gene in combination with amivantamab |

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| Registration date | Trade name | Manufacturer | INN | Pharmaceutical form | Class | Indication |
|---|---|---|---|---|---|---|
| Aug 14 | Niktimvo | Incyte Corporation | Axatilimab-csfr | Solution for intravenous administration | CSF-1 receptor blocking antibody | Chronic graft-versus-host disease |
| Aug 14 | Livdelzi | Gilead Sciences, Inc. | Seladelpar | Capsules for oral administration | Peroxisome proliferator-activated receptor delta agonist | Primary biliary cholangitis in combination with UDCA in adults with inadequate response to UDCA monotherapy |
| Aug 12 | Nemluvio | Galderma Laboratories | Nemolizumab-ilto | Solution for subcutaneous injection | Interleukin 31 receptor antagonist | Nodular prurigo |
| Aug 09 | Yorvipath | Ascendis Pharma Bone Diseases A/S | Palopegteriparatide | Solution for subcutaneous injection | Parathyroid hormone analog | Adult hypoparathyroidism |
| Aug 06 | Voranigo | Servier Pharmaceuticals LLC | Vorasidenib | Tablets for oral administration | Isocitrate dehydrogenase 1 and 2 inhibitor | Grade 2 astrocytoma or oligodendroglioma (diffuse forms) in adults and children over 12 years of age |
| Jul 25 | Leqselvi | Halo Pharmaceutical Inc. | Deuruxolitinib | Tablets for oral administration | Janus kinase inhibitor | Severe alopecia areata |
| Jul 02 | Kisunla | Eli Lilly and Company | Donanemab-azbt | Solution for intravenous administration | Monoclonal antibody to beta-amyloid | Alzheimer's disease |
| Jun 26 | Ohtuvayre | Verona Pharma, Inc. | Ensifentrine | Inhalation suspension | Phosphodiesterase 3 and 4 inhibitor | Chronic obstructive pulmonary disease |
| Jun 20 | Piasky | Genentech, Inc. | Crovalimab-akkz | Solution for intravenous or subcutaneous administration | Complement component C5 inhibitor | Complement-dependent intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria |
| Jun 18 | Sofdra | Botanix SB Inc. | Sofpironium | Topical gel | Anticholinergic | Primary axillary hyperhidrosis in adults and children 9 years of age and older |
| Jun 10 | Iqirvo | Ipsen Biopharmaceuticals, Inc. | Elafibranor | Tablets for oral administration | Peroxisome proliferator-activated receptor agonist | Primary biliary cholangitis in combination with UDCA in adults with inadequate response to UDCA monotherapy |

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| Registration date | Trade name | Manufacturer | INN | Pharmaceutical form | Class | Indication |
|---|---|---|---|---|---|---|
| Jun 06 | Rytelo | Geron Corporation | Imetelstat | Solution for intravenous administration | Telomerase oligonucleotide inhibitor | Low- and intermediate-risk myelodysplastic syndromes in adult patients with anemia requiring transfusions of 4 or more units of red blood cell mass within 8 weeks in case of ineffectiveness or impossibility of using erythropoiesis-stimulating agents |
| May 16 | Imdelltra | Amgen Inc. | Tarlatamab-dlle | Solution for intravenous administration | Bispecific delta-like ligand 3 (DLL3) targeting CD3-cell engager | Advanced small cell lung cancer at the time of progression or after platinum-based therapy in adults |
| Apr 26 | Xolremdi | X4 Pharmaceuticals, Inc. | Mavorixafor | Capsules for oral administration | CXC-chemokine receptor 4 antagonist | Increase in the number of mature neutrophils and lymphocytes in the peripheral blood in adults and children over 12 years of age with WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis) |
| Apr 23 | Ojemda | Day One Biopharmaceuticals, Inc. | Tovorafenib | Oral solution | Kinase inhibitor | Relapsed or refractory pediatric low-grade glioma in children 6 months and older |
| Apr 22 | Anktiva | Altor BioScience, LLC | Nogapendekin alfa inbakicept-pmln | Solution for intravesical administration | IL-15 agonist | Treatment of BCG-unresponsive non-muscle invasive bladder cancer with carcinoma *in situ* with or without papillary tumors in adults in combination with BCG vaccine |
| Apr 17 | Lumisight | Lumicell, Inc. | Pegulicianine | Solution for intravenous administration | Dye | Detection of cancerous tissue in the resection cavity after removal of the primary tumor during lumpectomy in adult patients with breast cancer |
| Apr 03 | Zevtera | Basilea Pharmaceutica International Ltd | Ceftobiprole medocaril sodium | Solution for intravenous administration | Cephalosporin | *Staphylococcus aureus* bacteremia, including right-sided infective endocarditis in adults; acute bacterial skin and skin structure infections in adults; community-acquired pneumonia in adults and children 3 months and older. |
| March 29 | Voydeya | Alexion Pharmaceuticals, Inc. | Danicopan | Tablets for oral administration | Complement factor D inhibitor | Add-on therapy to ravulizumab or eculizumab for extravascular hemolysis in adults with paroxysmal nocturnal hemoglobinuria. |
| March 27 | Vafseo | Akebia Therapeutics, Inc. | Vadadustat | Tablets for oral administration | Hypoxia-inducible factor prolyl hydroxylase inhibitor | Anemia due to chronic kidney disease in adults who have been on dialysis for at least 3 months |

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| Registration date | Trade name | Manufacturer | INN | Pharmaceutical form | Class | Indication |
|---|---|---|---|---|---|---|
| March 26 | Winrevair | Merck Sharp & Dohme LLC | Sotatercept-csrk | Solution for subcutaneous injection | Activin signaling inhibitor | Pulmonary arterial hypertension |
| March 21 | Duvyzat | ITF Therapeutics, LLC | Givinostat | Oral suspension | Histone deacetylase inhibitor | Duchenne muscular dystrophy in children aged 6 years and older |
| March 19 | Tryvio | Idorsia Pharmaceuticals US Inc | Aprocitentan | Tablets for oral administration | Endothelin receptor antagonist | Arterial hypertension in combination with other blood pressure-lowering drugs in adults when adequate blood pressure control cannot be achieved |
| March 14 | Rezdiffra | UPM Pharmaceuticals | Resmetirom | Tablets for oral administration | Thyroid hormone β agonist | Treatment (in conjunction with diet and exercise) of non-alcoholic steatohepatitis without cirrhosis with moderate to advanced fibrosis |
| March 13 | Tevimbra | BeiGene USA, Inc. | Tislelizumab-jsgr | Solution for intravenous administration | Antibody to programmed cell deathprotein | Unresectable or metastatic squamous cell carcinoma of the esophagus in adults after chemotherapy that did not contain PD-1 inhibitors or PD-L1 inhibitors |
| Feb 29 | Letybo | Hugel, Inc. | Letibotulinumtoxin A-wlbg | Solution for intramuscular injection | *Botulinum* toxin | Temporary improvement in the appearance of moderate to severe glabellar (between the eyebrows, on the forehead and above the nose) wrinkles |
| Feb 22 | Exblifep | Allecra Therapeutics SAS, 68300 Saint Louis, France | Cefepime and enmetazobactam | Solution for intravenous administration | Cephalosporin and beta-lactamase inhibitor | Complicated urinary tract infections |
| Jan 05 | Zelsuvmi | EPIH SPV, LLC | Berdazimer | Topical gel | NO-releasing agent | *Molluscum contagiosum* |

Note: HER — human epidermal growth factor receptor; PD — programmed death protein 1; HR — hormone receptor; IL — interleukin; INN — international nonproprietary name; NSCLC — non-small cell lung cancer; CSF-1 — colony-stimulating factor 1.

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## MATERIALS AND METHODS

The review describes drugs approved for use by the FDA. Data on the indications and mechanisms of action of drugs were taken from the Summary of Product Characteristics (SmPC) published by the FDA (https://www.fda.gov/) and supplemented with descriptions from the Drugs.com website. Structural formulas of drugs were taken from the PubChem resource. In cases where PubChem did not contain the required formula, the molecular structure was taken from the Drugs.com website or from the instructions for medical use of the medicine with this active substance. The ChemDraw program was used to unify the appearance of the formulas.

To update the literature data, a search for publications on preclinical and clinical studies of the medicine or its active substance, as well as publications on fundamental research, was carried out in the validated bibliographic database of the US National Library of Medicine (NLM) PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), on the ResearchGate (https://www.researchgate.net/) and Google Scholar (https://scholar.google.ru/) websites, as well as in Russian scientific online libraries (http://elibrary.ru and http://cyberleninka.ru/). Search queries included combinations of keywords in combination with pharmacological properties (for example, "arimoclomol in Niemann-Pick disease", etc.). Articles with a publication date no later than 2015 were used. For describing studies of fundamental mechanisms, no restrictions were placed on the publication date.

The review also presents data from the reports of the Center for Drug Evaluation and Research (CDER) "Advancing Health Through Innovation"[6, 7, 8, 9] for the periods from 2021 to 2024.

## RESULTS

The dynamics of CDER FDA registration are presented in Figure 1. The ratio of the number of medicines depending on their class is presented in Table 2. Figure 2 reflects the change in the proportion of drugs belonging to different segments and registration strategies.

[6] CDER. Advancing Health Through Innovation: New Drug Therapy Approvals 2021. Available from: https://www.fda.gov/media/155227/download?attachment

[7] CDER. Advancing Health Through Innovation: New Drug Therapy Approvals 2022. Available from: https://www.fda.gov/media/164429/download?attachment

[8] CDER. Advancing Health Through Innovation: New Drug Therapy Approvals 2023. Available from: https://www.fda.gov/media/175253/download?attachment

[9] CDER. Advancing Health Through Innovation: New Drug Therapy Approvals 2024. Available from: https://www.fda.gov/media/184967/download?attachment

**Table 2 – Distribution of drugs approved by the FDA in 2024 by groups depending on the nature and mechanism of action**

| Segment | Group | Subgroup | Quantity, *n* | Share of all registered, % | |
|---|---|---|---|---|---|
| Small molecules | Receptor ligands | | 9 | 18% | 60% |
| | Ligands | Kinase inhibitors | 5 | 10% | |
| | | Non-enzyme ligands | 5 | 10% | |
| | | Other enzyme inhibitors | 7 | 14% | |
| | Antibiotics | | 4 | 8% | |
| Biologics | Peptides, proteins and oligonucleotides | | 5 | 10% | 34% |
| | mAb | Antitumor | 6 | 12% | |
| | | Anti-inflammatory | 3 | 6% | |
| | | Other | 3 | 6% | |
| Imaging agents | | | 3 | 6% | 6% |

Note: mAb — monoclonal antibody.

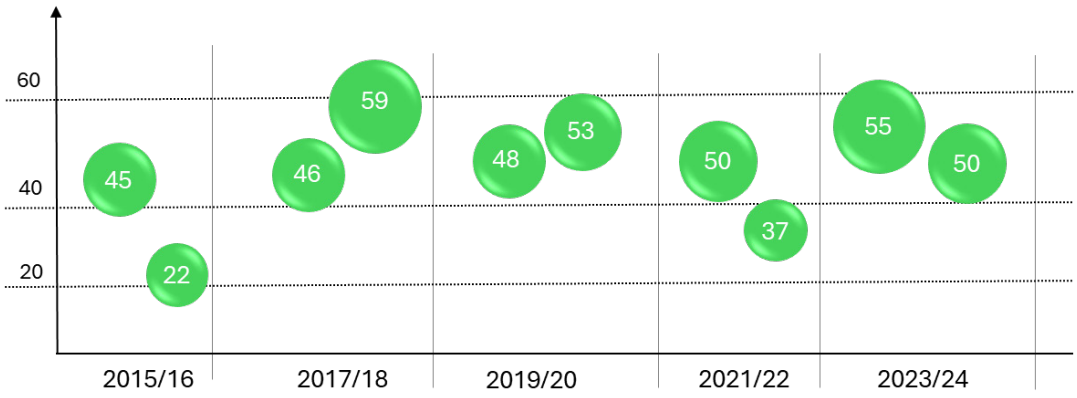


**Figure 1 – Number of medicines registered with the CDER from 2015 to 2024**
Note: the X-axis represents years, the Y-axis represents the number of registered medicines.

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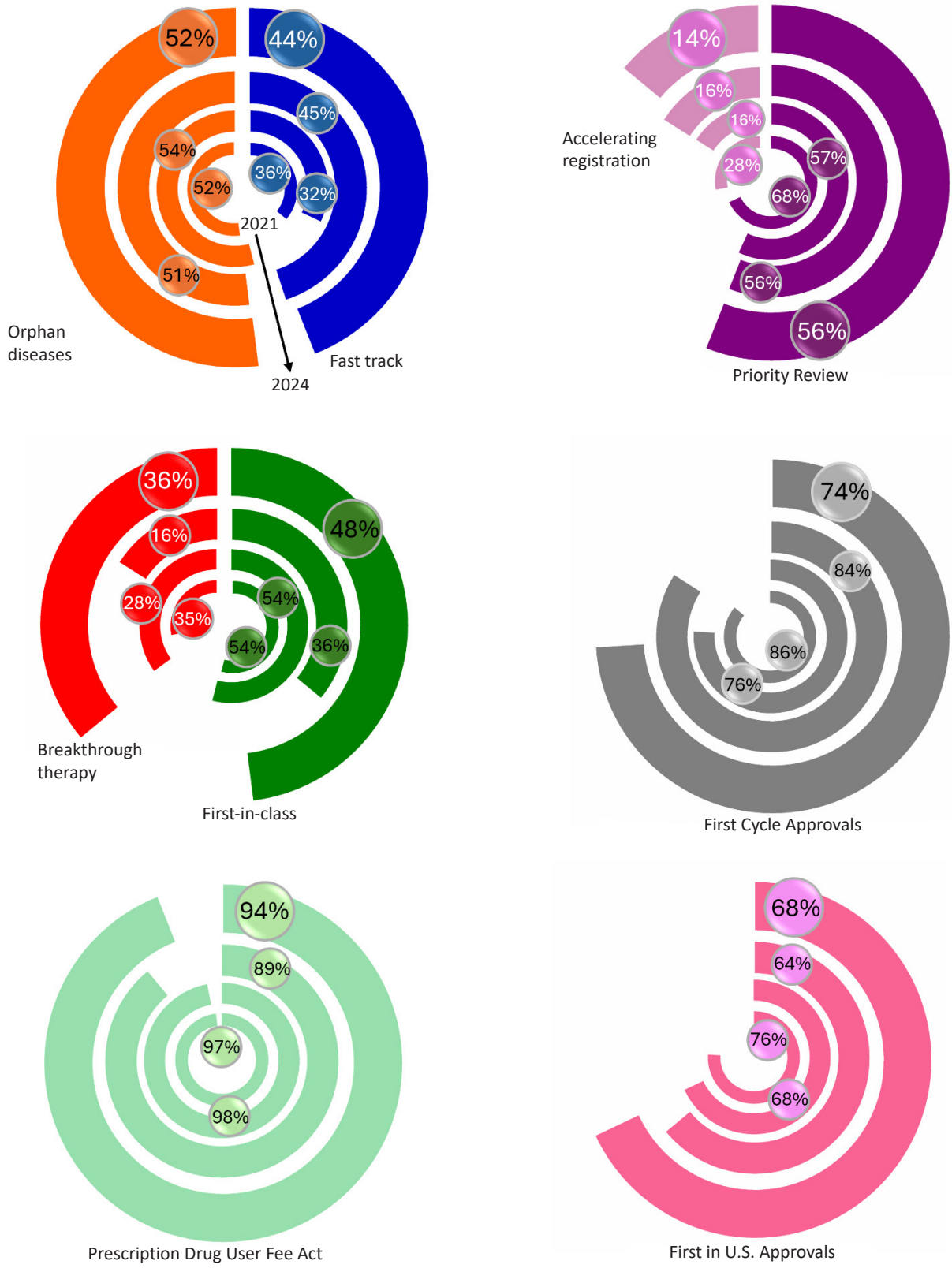


**Figure 2 – Shares of original medicines from 2021 to 2024 by market segment**
Note: data are presented as the proportion of drugs in the specified segment from the total number of drugs registered for the specified year.

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The descriptions and structural formulas of the original medicines registered in 2024 are presented below.

## Small Molecules

### Receptor Ligands

*Crinecerfont*

Crinecerfont (CRENESSITY™, capsules for oral administration or solution for oral administration) is a selective corticotropin-releasing hormone (CRH) receptor type 1 antagonist used as an adjunct to glucocorticoid replacement therapy to control androgens in adults and children aged 4 years and older with classic congenital adrenal hyperplasia. Crinecerfont (Fig. 3A) blocks the binding of CRH to the CRH type 1 receptor, but not to the type 2 receptor, which leads to suppression of adrenocorticotropic hormone secretion from the pituitary gland, resulting in a decrease in adrenal androgen production[10, 11].

*Landiolol*

Landiolol (RAPIBLYK, solution for intravenous administration) is a selective β1-adrenergic receptor antagonist used for short-term reduction of ventricular rate in adult patients with supraventricular tachycardia, including atrial fibrillation or flutter. Landiolol (Fig. 3B) suppresses the positive chronotropic effects of catecholamines (adrenaline and noradrenaline) on the heart. Landiolol does not exhibit membrane-stabilizing activity or intrinsic sympathomimetic activity at the recommended dosage *in vitro*[12, 13].

*Aprocitentan*

Aprocitentan (TRYVIO™, tablets for oral administration) is an endothelin receptor antagonist used to treat arterial hypertension in combination with other medicines that lower blood pressure in adults when adequate blood pressure control cannot be achieved. Aprocitentan (Fig. 4A) binds to endothelin 1 receptors A and B and prevents the development of the latter's pathogenetics: endothelial dysfunction, hypertrophy and vascular remodeling, as well as sympathetic activation of aldosterone synthesis[14, 15].

*Sofpironium*

Sofpironium (SOFDRA™, gel for topical use) is an anticholinergic medicine intended for the treatment of primary axillary hyperhidrosis in adults and children over 9 years of age. It is a competitive inhibitor of acetylcholine receptors located in some peripheral tissues (including axillary sweat glands). Sofpironium (Fig. 4B) has an indirect effect on excessive sweating, preventing the activation of acetylcholine receptors[16, 17].

*Seladelpar*

Seladelpar (LIVDELZI®, capsules for oral administration) is an agonist of the peroxisome proliferator-activated receptor delta (PPAR) δ, intended for the treatment of primary biliary cholangitis in combination with ursodeoxycholic acid (UDCA) in adults with insufficient efficacy of UDCA as monotherapy. The mechanism by which seladelpar (Fig. 5A) exerts its therapeutic effect in patients with primary biliary cholangitis has not been well studied. The therapeutic effect includes inhibition of bile acid synthesis through activation of PPARδ, which is a nuclear receptor expressed in most cells, including hepatocytes. Seladelpar activates PPARδ, which leads to a decrease in bile acid synthesis activity by suppressing cytochrome P450 (CYP) 7A1 via a fibroblast growth factor 21 (FGF21)-dependent mechanism. CYP7A1 is a key enzyme in the synthesis of bile acids from cholesterol. The indication for the use of seladelpar was established based on its ability to reduce alkaline phosphatase activity. The effect on survival or prevention of liver function decompensation has not been proven[18, 19].

[10] Drugs. com. Crenessity. Available from: https://www.drugs.com/crenessity.html
[11] CRENESSITY. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/218808s000,218820s000lbl.pdf
[12] Drugs. com. Rapiblyk. Available from: https://www.drugs.com/rapiblyk.html
[13] RAPIBLYK. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217202s000lbl.pdf
[14] Drugs. com. Tryvio. Available from: https://www.drugs.com/tryvio.html
[15] TRYVIO. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217686s000lbl.pdf
[16] Drugs. com. Sofdra. Available from: https://www.drugs.com/sofdra.html
[17] SOFDRA. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217347s000lbl.pdf
[18] Drugs. com. Livdelzi. Available from: https://www.drugs.com/livdelzi.html
[19] LIVDELZI. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217899s000lbl.pdf

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*Elafibranor*

Elafibranor (IQIRVO®, oral tablets) is a PPAR agonist indicated for the treatment of primary biliary cholangitis in combination with UDCA in adults with inadequate response to UDCA as monotherapy. In vitro, elafibranor (Fig. 5B) has affinity for PPARα, PPARγ, and PPARδ. However, the mechanism of this drug in patients with primary biliary cholangitis has not been established. It is assumed that the therapeutic effect is mediated by inhibition of bile acid synthesis, which, in turn, is regulated by PPARα and PPARδ[20, 21].

*Xanomeline+trospium chloride*

Xanomeline and trospium chloride (COBENFY™, oral capsules) is a combination of a muscarinic receptor agonist and antagonist with antipsychotic activity, indicated for the treatment of schizophrenia in adults. The exact mechanism of action of the combination is unknown. Xanomeline (Fig. 6A) binds to muscarinic receptors. The Ki of xanomeline for binding to the M1 receptor is 10 nmol/L, for binding to M2 — 12 nmol/L, to M3 — 17 nmol/L, to M4 — 7 nmol/L, and for binding to M5 — 22 nmol/L. Thus, xanomeline has the most pronounced agonistic effect on the M1 and M4 receptors. Trospium chloride (Fig. 6B) is a muscarinic receptor antagonist that acts primarily in the tissues of the peripheral nervous system. The combination of these compounds is the first antipsychotic medicine whose action is based on interaction with cholinergic receptors, rather than with dopamine receptors, which was the basis of the action of medicines that have long served as the standard of treatment[22, 23].

*Mavorixafor*

Mavorixafor (XOLREMDI™, oral capsules) is an antagonist of receptor 4 to CXC-chemokine, used in adults and children over 12 years of age with WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis) to increase the number of mature neutrophils and lymphocytes in peripheral blood. Mavorixafor (Fig. 7A) is a *CXCR4* antagonist that prevents the binding of the stromal cell factor 1α ligand (stromal-derived factor-1α [SDF-1α]/CXC Chemokine Ligand 12 [CXCL12] SDF-1/CXCL12). This ligand modulates the transport of lymphocytes from the bone marrow to the blood and back. Functional mutations in the *CXCR4* gene, which are found in patients with WHIM syndrome, lead to increased sensitivity to *CXCL12* and retention of leukocytes in the bone marrow. Mavorixafor inhibits the interaction of *CXCL12* with *CXCR4*, both with the mutant form and with the wild-type form. The use of mavorixafor leads to the mobilization of neutrophils and lymphocytes from the bone marrow into the peripheral blood[24, 25].

*Resmetirom*

Resmetirom (REZDIFFRA, oral tablets) is a thyroid hormone receptor beta (THR-β) agonist indicated in combination with diet and exercise for the treatment of non-alcoholic steatohepatitis without cirrhosis with moderate to severe fibrosis (Stage F2–F3). The use of resmetirom in patients with decompensated cirrhosis is contraindicated. Resmetirom (Fig. 7B) is a partial THR-β agonist, causing an effect that is 83.8% of that developing in response to triiodothyronine exposed to THR-β. Since THR-β is the main form of the thyroid hormone receptor in the liver, the main effect of the medicine is to reduce the concentration of triglycerides in the liver[26, 27].

*Berdazimer*

Berdazimer (ZELSUVMI™, topical gel) is a nitric oxide (NO) releasing agent used to treat molluscum contagiosum. Its action is associated with the release of NO, which is believed to help fight the virus, although the exact mechanism is not fully understood. Berdazimer is a polymer formed from sodium 1-hydroxy-3-methyl-3-(3-(trimethoxysilyl)propyl)-1-triazene-2-oxide and tetraethyl silicate. The structural formula is shown in Figure 8[28, 29].

[20] Drugs. com. Iqirvo. Available from: https://www.drugs.com/iqirvo.html
[21] IQIRVO. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/218860s000lbl.pdf
[22] Drugs. com. Cobenfy. Available from: https://www.drugs.com/cobenfy.html
[23] COBENFY. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/216158s000lbl.pdf
[24] Drugs.com. Xolremdi. Available from: https://www.drugs.com/xolremdi.html
[25] XOLREMDI. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/218709s000lbl.pdf
[26] Drugs.com. Rezdiffra. Available from: https://www.drugs.com/rezdiffra.html
[27] REZDIFFRA. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217785s000lbl.pdf
[28] Drugs.com. Zelsuvmi. Available from: https://www.drugs.com/zelsuvmi.html
[29] ZELSUVMI. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217424s000lbl.pdf

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## Ligands of enzymes and other proteins

### Kinase inhibitors

*Ensartinib*

Ensartinib (ENSACOVE™, oral capsules) is an anaplastic lymphoma kinase (ALK) inhibitor that also suppresses the activity of other kinases, including MET and ROS1. Ensartinib (Fig. 9A) is indicated for adult patients with locally advanced or metastatic ALK-positive NSCLC who have not previously received ALK inhibitors. In vitro, ensartinib inhibited ALK phosphorylation, leading to blockade of downstream signaling pathways, thereby preventing proliferation in cells containing ALK fusion proteins and its mutated forms. In vivo, ensartinib had an antitumor effect in an NSCLC xenograft (ALK fusion) in mice[30, 31].

*Inavolisib*

Inavolisib (ITOVEBI, oral tablets) is a phosphatidylinositol 3-kinase (PI3K) inhibitor, predominantly active against PI3Kα. Inavolisib (Fig. 9B) is used to treat locally advanced or metastatic breast cancer, provided that it is endocrine-resistant, has a PIK3CA mutation, is HR-positive, HER2-negative after recurrence, during or after completion of adjuvant endocrine therapy[32, 33].

*In vitro*, the medicine induces degradation of the p110α subunit, mutated PI3K, inhibits phosphorylation and the protein kinase B (AKT) cascade, leading to a decrease in cell proliferation and apoptosis of breast cancer cells with the *PIK3CA* mutation. In vivo, inavolisib inhibits the growth of breast cancer xenografts in mice. The combination of inavolisib with palbociclib and fulvestrant inhibits tumor growth more significantly than each of the drugs separately.

*Lazertinib*

Lazertinib (LAZCLUZE™, oral tablets) is an epidermal growth factor receptor (EGFR) kinase inhibitor intended for the treatment of NSCLC. The medicine (Fig. 9B) suppresses EGFR activity at lower concentrations than when exposed to the wild-type receptor. In NSCLC cells and in mouse xenografts of these cells with exon 19 deletion or L858R substitution in exon 21, lazertinib has antitumor activity. In similar models, lazertinib enhances the antitumor effect of amivantamab[34, 35].

*Tovorafenib*

Tovorafenib (OJEMDA, oral solution) is a kinase inhibitor (rapidly accelerated fibrosarcoma RAF) type II kinases, B form of RAF kinase (BRAF) V600E mutation, wild-type BRAF, and wild-type CRAF. This medicine is used to treat relapsed or refractory pediatric low-grade glioma in children older than 6 months. Tovorafenib (Fig. 11A) had antitumor activity in animals with a tumor xenograft carrying mutations provoking fibrosarcoma with BRAF mutations[36, 37].

*Deucravacitinib*

Deucravacitinib (LEQSELVI™, oral tablets) is a Janus kinase (JAK) inhibitor intended for the treatment of adults with severe alopecia areata. JAK regulates the signaling pathways of a number of cytokines and growth factors that play an important role in hematopoiesis and immunity. JAK signaling involves the activation of signal transducers and activators of transcription to cytokine receptors, which leads to modulation of the expression of certain genes. In vitro, deucravacitinib (Fig. 10A) inhibited JAK1 and JAK2 more significantly than JAK3. The relationship between JAK inhibition and the therapeutic activity of deucravacitinib has not been fully studied[38, 39].

### Other enzyme inhibitors

*Vorasidenib*

Vorasidenib (VORANIGO®, oral tablets) is an isocitrate dehydrogenase (IDH) 1 and IDH2 inhibitor intended for the treatment of grade 2 astrocytoma or oligodendroglioma (diffuse forms) in adults and children 12 years and older. Voracidenib in medicinal forms is used as a co-crystal of hemihydrate and hemicitric acid (Fig. 11B). *In vitro*, voracidenib suppresses the activity of wild-type and mutant variants of IDH1,

[30] Drugs.com. Ensacove. Available from: https://www.drugs.com/ensacove.html
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[33] ITOVEBI. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/219249s001lbl.pdf
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including forms with R132H substitution. In animal models using tumors expressing mutant IDH1 and IDH2, administration of vorasidenib reduced the production of 2-hydroxyglutarate and partially normalized impaired cell differentiation[40, 41].

*Ensifentrine*

Ensifentrine (OHTUVAYRE, inhalation suspension) is a phosphodiesterase (PDE) 3 and 4 inhibitor used to treat chronic obstructive pulmonary disease. PDE3 predominantly hydrolyzes cAMP and has the ability to hydrolyze cGMP, while PDE4 hydrolyzes only cGMP. Ensifentrine (Fig. 10B) inhibits the activity of PDE3 and PDE4, which leads to the accumulation of intracellular cAMP and cGMP and, as a result, to the suppression of intracellular signal transduction[42, 43].

*Imetelstat*

Imetelstat (RYTELO, solution for intravenous administration) is an oligonucleotide telomerase inhibitor intended for the treatment of myelodysplastic syndromes with low and intermediate risk in adult patients with anemia requiring transfusions of 4 or more units of red blood cell mass within 8 weeks with ineffectiveness or impossibility of using erythropoiesis-stimulating agents. Imetelstat (Fig. 12) inhibits human telomerase by binding to the template region of its RNA component, which leads to suppression of the activity of this enzyme and prevention of telomere elongation. Increased activity and expression of RNA reverse transcriptase of telomerase was found in myelodysplastic syndromes, in cancer stem and progenitor cells. According to the results of preclinical studies, imetelstat reduced telomere length, suppressed the proliferation of malignant stem and progenitor cells, and induced apoptosis[44, 45].

*Givinostat*

Givinostat (DUVYZAT, suspension for oral administration; Fig. 13) is a histone deacetylase inhibitor used to treat Duchenne muscular dystrophy in children aged 6 years and older. The mechanism of alleviation is unknown. In a study involving children who were given the drug for 18 months, it was noted that the increase in the fraction of fat in the lateral broad muscle of the thigh was 7.48 *vs* 10.89% (in the group of patients using placebo)[46, 47].

*Vadadustat*

Vadadustat (VAFSEO®, tablets for oral administration) is an inhibitor of hypoxia-inducible prolyl hydroxylase (HIF-prolyl-4-hydroxylases, PH) intended for the treatment of anemia caused by chronic kidney disease in adults who have been on dialysis for at least 3 months. Vadadustat (Fig. 14A) is a reversible inhibitor of PH1, PH2 and PH3. Due to this activity, the use of vadadustat leads to stabilization and accumulation of transcription factors 1α and 2α induced by hypoxia, as well as an increase in the production of erythropoietin[48, 49].

*Danicopan*

Danicopan (VOYDEYA™, tablets for oral administration) is an inhibitor of factor D of the complement system, intended for additional therapy to ravulizumab or eculizumab for extravascular hemolysis in adults with paroxysmal nocturnal hemoglobinuria[50, 51].

In paroxysmal nocturnal hemoglobinuria, intravascular hemolysis occurs with the participation of a membrane-attacking complex, and the development of extravascular hemolysis is enhanced by opsonization with the participation of complement system fragment C3. Danicopan prevents the development of extravascular hemolysis, while ravulizumab or eculizumab prevents intravascular hemolysis.

Danicopan (Fig. 14B), reversibly binding to factor D (adipsin, C3 proactivator convertase) of the complement system, inhibits the alternative pathway of its activation. The effect of danicopan on factor D

[40] Drugs.com. Voranigo. Available from: https://www.drugs.com/voranigo.html
[41] VORANIGO. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/218784s000lbl.pdf
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[48] Drugs.com. Vafseo. Available from: https://www.drugs.com/vafseo.html
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prevents the cleavage of factor B into Ba and Bb, which are necessary for the formation of C3 component convertase and activation of subsequent effectors of the complement system, including C3 opsonization.

### Non-enzyme ligands

*Vanzacaftor+tezacaftor+deutivacaftor*

Vanzacaftor, tezacaftor and deutivacaftor (ALYFTREK, tablets for oral administration) is a combination of cystic fibrosis transmembrane regulator (*CFTR*) ligands intended for the treatment of cystic fibrosis in patients aged 6 years and older with at least one F508del mutation or another mutation in the *CFTR* gene. The structural formulas of the components included in the drug product are shown in Figure 15. Vanzacaftor and tezacaftor bind to different regions of *CFTR* and additively contribute to the processing and expression of mutant forms of *CFTR* on the cell surface. Deutivacaftor increases the probability of opening the *CFTR* channel on the cell surface. Together, these 3 molecules enhance *CFTR* activity, which leads to increased chloride transport across the cell membrane and alleviation of cystic fibrosis[52, 53].

*Revumenib*

Revumenib (REVUFORJ, tablets for oral administration) is a menin inhibitor used to treat relapsed or refractory acute leukemia with translocation of the lysine methyltransferase 2A (histone-lysine N-methyltransferase 2A, *KMT2A*) gene in children from 1 year and adults. Revumenib (Fig. 16G) blocks the interaction of *KMT2A* and the *KMT2A*-menin hybrid protein. Binding of the *KMT2A*-menin hybrid protein is involved in the mechanism of reorganization of acute leukemia under the control of *KMT2A*, which occurs after activation of leukemogenic transcription. In preclinical studies, suppression of the interaction of menin and *KMT2A* in cells expressing *KMT2A* hybrid proteins with revumenib led to a change in the transcription of a number of genes, including differentiation markers. Revumenib had an antiproliferative and antitumor effect *in vitro* and *in vivo* against cells containing *KMT2A* hybrid proteins[54, 55].

*Acoramidis*

Acoramidis (ATTRUBY™, tablets for oral administration) is a transthyretin stabilizer used to treat transthyretin amyloid cardiomyopathy (cardiomyopathy of transthyretin-mediated amyloidosis ATTR-CM), with wild-type or variant form of the transthyretin gene in adults to reduce mortality and hospitalizations due to cardiovascular disorders. Acoramidis (Fig. 16A) is a selective transthyretin stabilizer. By binding to transthyretin at the thyroxine binding site, acoramidis slows down the dissociation of the transthyretin tetramer, which is the limiting stage of amyloidogenesis[56, 57].

### Drugs for the treatment of Niemann-Pick disease type C

*Levacetylleucine*

Levacetylleucine (AQNEURSA™, suspension for oral administration) is a leucine derivative used to treat Niemann-Pick disease type C (NPC1 or NPC2 mutation, cell membrane proteins) in children with a body weight >15 kg and in adults. The mechanism of action of levacetylleucine (Fig. 16B) is unknown[58, 59].

*Arimoclomol*

Arimoclomol (MIPLYFFA, capsules for oral administration) is an experimental compound used to treat Niemann-Pick disease type C (NPC1 or NPC2 mutation, cell membrane proteins) in adults and children over 2 years of age. The mechanism of action of arimoclomol (Fig. 16C) is unknown[60, 61].

A clinical study was conducted involving 50 patients aged 2 to 18 years suffering from Niemann-Pick disease type C. Participants took arimoclomol at a dose of 16, 31 or 62 mg orally in capsules 3 times a day, or placebo. The primary endpoint was the change in the score on the five-structure scale of Niemann-Pick disease type C (Niemann-Pick Disease Type C Clinical Severity Scale, NPCCSS) from the start of the study to 12 months. As a result, it was found that arimoclomol

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[55] REVUFORJ https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/218944s000lbl.pdf
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[60] Drugs.com. Miplyffa. Available from: https://www.drugs.com/miplyffa.html
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significantly slowed the progression of the disease. The average decrease in progression in patients taking arimoclomol was 0.76 versus 2.15 in patients taking placebo. The difference in progression (estimated through statistical analysis) was approximately 1.40, which is significant and indicates a decrease in the rate of the disease. Side effects occurred in 88% of treated patients, but there were fewer serious complications — 14.7 *vs* 31.3% in patients taking placebo [6].

### Antibiotics

*Ceftobiprole medocaril sodium*

Ceftobiprole medocaril sodium (ZEVTERA, solution for intravenous administration) is a cephalosporin used to treat:

- *Staphylococcus aureus* bacteremia, including right-sided infectious endocarditis in adults;
- Acute bacterial infections of the skin and skin structures in adults;
- Community-acquired pneumonia in adults and children over 3 months[62, 63].

The antibacterial activity of ceftobiprole (Fig. 17) is mediated by the suppression of bacterial wall synthesis. In vitro, ceftobiprole was active against both gram-positive and gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA)[64].

Bactericidal activity is justified by the binding of the drug to penicillin binding protein (PBP) and inhibition of their transpeptidase activity, which is necessary for the synthesis of the peptidoglycan layer of the bacterial cell wall. Ceftobiprole has a high affinity for PBP 1–4 *Staphylococcus aureus*, including penicillin-resistant *Streptococcus pneumoniae*.

Ceftobiprole is not active against bacteria producing ESBL, TEM, SHV or CTX-M families, as well as against serine carbapenemases, metallo-β-lactamases of classes B and C (AmpC). No cross-resistance of ceftobiprole and antibiotics of other classes has been identified. Resistance may be present in strains resistant to cephalosporins.

*Cefepime+enmetazobactam*

Cefepime and enmetazobactam (EXBLIFEP®, solution for intravenous administration) is a combination of a cephalosporin and a β-lactamase inhibitor used to treat complicated urinary tract infections. Cefepime (Fig. 18A), which is part of the drug product, belongs to β-lactam antibiotics of the cephalosporin group IV generation. The chemical structure includes β-lactam and iminotetrahydrothiazine rings, as well as an N-methylpyrrolidine side chain, which improves penetration through bacterial walls and binding to PBP. Enmetazobactam (Fig. 18B) is a β-lactamase inhibitor that protects cefepime from cleavage by some serine β-lactamases, such as ESBL[65, 66].

The spectrum of antibacterial activity of the medicine EXBLIFEP® is presented in Table 3. Mechanisms of resistance include: modification of PBP, increased production of β-lactamases resistant to enmetazobactam, increased production of efflux pumps, as well as mutations of the membrane porin.

**Table 3 – Spectrum of antibacterial activity of the combination of cefepime and enmetazobactam[67]**

| | |
|---|---|
| Clinically proven effectiveness | |
| Gram-negative bacteria | *Escherichia coli* |
| | *Klebsiella pneumoniae* |
| | *Pseudomonas aeruginosa* |
| | *Proteus mirabilis* |
| | *Enterobacter cloacae* |
| Efficacy confirmed *in vitro*, but there is no data on clinical significance | |
| Gram-negative bacteria | *Citrobacter freundii* |
| | *Klebsiella aerogenes* |
| | *Klebsiella oxytoca* |
| | *Providencia stuartii* |
| | *Providencia rettgeri* |
| | *Serratia marcescens* |

*Sulopenem etzadroxil+probenecid*

Sulopenem etzadroxil and probenecid (ORLYNVAH™, tablets for oral administration) is a combined medicine of a tubular transport inhibitor and an antibiotic recommended for the treatment of urinary tract infections caused by *Escherichia coli*, *Klebsiella pneumoniae* or *Proteus mirabilis*. Probenecid (Fig. 19A) reduces the clearance of sulopenem (Fig. 19B) by suppressing its excretion through OAT3, which leads

[62] Drugs.com. Zevtera. Available from: https://www.drugs.com/zevtera.html
[63] ZEVTERA. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/218275s000lbl.pdf
[64] PubChem. Ceftobiprole. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/135413542
[65] Drugs.com. Exblifep. Available from: https://www.drugs.com/exblifep.html
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to an increase in the concentration of the antibiotic in the blood plasma[68, 69, 70].

For sulopenem, as for other β-lactam antibiotics, a correlation has been proven between the duration of the drug in plasma, at a concentration above MIC, and antimicrobial activity, which justifies the combination with a renal excretion inhibitor.

Sulopenem etzadroxil is a prodrug. Sulopenem *in vitro* is active against gram-positive and gram-negative aerobes and anaerobes. The antimicrobial activity of sulopenem is mediated by its ability to suppress cell wall synthesis, as well as by the binding of the drug product to PBP. The binding affinity of sulopenem to PBP in *Escherichia coli* is in the following order: PBP2 >PBP1A >PBP1B >PBP4 >PBP3 >PBP5/6.

Factors of bacterial resistance to sulopenem may be extended-spectrum β-lactamases (ESBL), including carbapenemases. Changes in PBP, an increase in the number of efflux pumps and a decrease in the number of porins on the outer membrane also affect. Sulopenem is active against *Enterobacterales* expressing some ESBL, for example, AmpC, CTX-M, TEM, SHV. Lines resistant to sulopenem were selected *in vitro* with a frequency of $1\times10^{-8}$.

## Biologics

### Peptides, proteins and oligonucleotides

*Olezarsen*

Olezarsen (TRYNGOLZA, solution for subcutaneous injection) is an antisense oligonucleotide directed against the apolipoprotein C-III (APOC-III) gene and indicated as an adjunct to diet to reduce triglyceride concentrations in adults with familial chylomicronemia syndrome. Olezarsen (Fig. 20) is an ASO-GalNAc3 conjugate that binds to apolipoprotein C-III mRNA, which leads to its degradation and a decrease in APOC-III concentration in blood serum. A decrease in APOC-III concentration leads to an increase in the clearance of triglycerides and very low density lipoproteins[71, 72].

*Nogapendekin alfa inbakicept-pmln*

Nogapendekin alfa inbakicept-pmln (ANKTIVA®, solution for intravesical administration) is an IL-15 receptor agonist used in combination with Bacillus Calmette-Guerin (BCG) vaccine for the treatment of BCG-unresponsive invasive bladder cancer with carcinoma in situ in adults with or without papillomas. IL-15 transmits signals through a heterotrimeric receptor consisting of γ-chain, β-chain and IL-15-specific α-subunit. On the surface of CD4+ and CD8+ T-cells, as well as on the surface of natural killers (NK), IL-15 interaction is carried out through the combined IL-2/IL-15 receptor. Binding of nogapendekin alfa inbakicept-pmln to this receptor leads to proliferation and activation of NK cells, CD8+-cells and memory cells, without activating the proliferation of regulatory T-cells. In vivo, intravesical administration of the drug product alone or in combination with BCG led to the development of an antitumor effect in a rat bladder cancer model induced by a carcinogen[73, 74].

*Palopegteriparatide*

Palopegteriparatide (YORVIPATH®, solution for subcutaneous injection) is a structural analogue of parathyroid hormone (amino acid sequence from 1 to 34, PTH[1-34]) intended for the treatment of hypoparathyroidism in adults. The structure of palopegteriparatide is shown in Figure 21. Under physiological conditions, palopegteriparatide releases PTH(1-34) with the achievement of prolonged systemic exposure. Endogenous PTH regulates extracellular calcium homeostasis in blood serum and reduces the concentration of phosphate in it. These effects of PTH are mediated by interaction with bone tissue and mobilization of calcium and phosphate in it, as well as stimulation of renal reabsorption of calcium and excretion of phosphates. Like endogenous PTH, PTH(1-34) released from palopegteriparatide has a parathyroid effect through the parathyroid hormone receptor 1, expressed on the surface of osteoblasts, osteocytes, renal tubule cells and in some other tissues[75, 76].

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[69] Drugs.com. Sulopenem etzadroxil and probenecid (Monograph). Available from: https://www.drugs.com/monograph/sulopenem-etzadroxil-and-probenecid.html
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*Sotatercept-csrk*

Sotatercept-csrk (WINREVAIR™, solution for subcutaneous injection) is an activin signaling inhibitor used to treat pulmonary arterial hypertension. Chemically, sotatercept is a homodimeric recombinant hybrid protein consisting of the extracellular domain of the human activin receptor type IIA (ActRIIA) and the Fc domain of human immunoglobulin (Ig) G1 associated with it. It binds to activin A and other ligands of the TGF-β superfamily. As a result of this interaction, sotatercept normalizes the balance of proproliferative and antiproliferative signaling pathways that modulate angiogenesis. In studies using rats with experimental pulmonary arterial hypertension, it was noted that an analogue of sotatercept reduced inflammation and suppressed the proliferation of endothelial and smooth muscle cells in case of vascularization disorders. This effect led to the cessation of right ventricular remodeling and improved hemodynamics[77, 78].

*Letibotulinumtoxin A-wlbg*

Letibotulinumtoxin A-wlbg (LETYBO, solution for intramuscular injection) is a modified botulinum toxin, an inhibitor of acetylcholine release and a blocker of neuromuscular transmission, intended for temporary improvement of the appearance of glabellar (between the eyebrows, on the forehead and above the nose) wrinkles of moderate to severe severity. Letibotulinumtoxin A-wlbg, when administered intramuscularly, penetrates into the nerve ending, cleaves the SNAP25 protein, which is necessary for the release of acetylcholine into the synaptic cleft, which leads to a dose-dependent decrease in muscle function. Restoration of muscle function occurs gradually due to the degradation of the neurotoxin and the formation of axonal processes. Reinnervation of muscles occurs, which leads to a slow elimination of the pharmacological effects of letibotulinumtoxin A-wlbg[79, 80].

## Monoclonal antibodies of antitumor action

Most registered mAb medicines are prescribed for the treatment of malignant neoplasms.

*Cosibelimab-ipdl*

Cosibelimab-ipdl (UNLOXCYT, solution for intravenous administration) is an antibody blocking the programmed death receptor-1 (PD-1) ligand, intended for the treatment of adult patients with metastatic or locally advanced squamous cell skin cancer who cannot undergo radiation therapy or surgical treatment.

The PD-1 ligand is expressed on tumor and immune cells infiltrating the tumor. This suppresses antitumor signals in the tumor microenvironment. Binding of the ligand to PD-1 and B7.1 on the surface of T-cells and antigen-presenting cells suppresses cytostatic activity, proliferation and cytokine production by T-lymphocytes. Cosibelimab binds to the PD-1 ligand and, thus, blocks the interaction between it and PD-1 and B7.1. This effect weakens the inhibitory effect of the PD-1 ligand on the antitumor response. Cosibelimab causes ADCC *in vitro*[81, 82].

*Zenocutuzumab-zbco*

Zenocutuzumab-zbco (BIZENGRI®, solution for intravenous administration) is a bispecific antibody to HER2, HER3, intended for the treatment of:

- adults with advanced unresectable or metastatic NSCLC, carriers of the neuregulin 1 (*NRG1*) gene fusion, provided the disease progresses during or after systemic therapy;
- adults with advanced, unresectable or metastatic pancreatic adenocarcinoma containing the NRG1 gene fusion, provided the disease progresses during or after systemic therapy.

Zenocutuzumab-zbco binds to the extracellular domains of HER2 and HER3 expressed on the surface of cells, including tumor cells, suppressing HER2:HER3 dimerization and preventing *NRG1* binding to HER3. This leads to a decrease in proliferation and signal transduction involving the PI3K-AKT-mammalian target of rapamycin (mTOR). In addition, zenocutuzumab-zbco induces ADCC. In studies on mouse models, zenocutuzumab-zbco showed antitumor activity in *NRG1* fusions in lung and pancreatic cancer[83, 84].

[77] Drugs.com. Winrevair. Available from: https://www.drugs.com/winrevair.html
[78] WINREVAIR. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/761363s000lbl.pdf
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[81] Drugs.com. Unloxcyt. Available from: https://www.drugs.com/unloxcyt.html
[82] UNLOXCYT. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/761297s000lbl.pdf
[83] Drugs.com. Bizengri. Available from: https://www.drugs.com/bizengri.html
[84] BIZENGRI. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/761352s001lbl.pdf

*Zanidatamab-hrii*

Zanidatamab-hrii (ZIIHERA®, solution for intravenous administration) is a bispecific antibody directed to HER2, used to treat adult patients with previously treated, unresectable or metastatic bile duct tumor positive for HER2 mutation (IHC 3+). The antibody binds to two extracellular regions of HER2, which leads to internalization (immersion of the receptor inside the cell) and a decrease in HER2 on the surface of tumor cells. Zanidatamab-hrii activates complement-mediated cytotoxicity, antibody-dependent cytotoxicity and antibody-dependent cellular phagocytosis. All these mechanisms led to the suppression of tumor growth and cell death *in vitro* and *in vivo*[85, 86].

*Zolbetuximab-clzb*

Zolbetuximab-clzb (VYLOY®, solution for intravenous administration) is a chimeric (human/mouse) antibody that, in combination with claudin 18.2 (CLDN18.2), causes antigen- and complement-dependent cytolysis of cells expressing CLDN18.2. Zolbetuximab-clzb enhances the antitumor activity of chemotherapeutic agents in a mouse tumor model expressing CLDN18.2. The "exposure-response" relationship, in relation to the efficacy and safety of the recommended doses of zolbetuximab-clzb in patients with locally advanced unresectable or metastatic HER2-negative CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma, has not been fully studied[87, 88].

*Tislelizumab-jsgr*

Tislelizumab-jsgr (TEVIMBRA™, solution for intravenous administration) is an antibody that blocks PD-1, used to treat unresectable or metastatic squamous cell carcinoma of the esophagus in adults after chemotherapy that did not contain PD-1 inhibitors or PD-1 ligand inhibitors. Binding of *PD-1* located on the surface of T-cells to PD-L1 and PD-L2 ligands leads to a decrease in T-cell proliferation and cytokine production. Upregulation of PD-L-dependent signaling pathways occurs in some tumors, which leads to suppression of immune surveillance of T-cells over these tumors. Tislelizumab-jsgr, binding to *PD-1*, blocks its interaction with PD-L1 and PD-L2, which allows the development of an antitumor immune response. In in vivo experiments using transgenic mice carrying the human PD-1 gene with tumor xenografts, tislelizumab suppressed tumor growth[89, 90].

*Crovalimab-akkz*

Crovalimab-akkz (PIASKY, solution for intravenous or subcutaneous administration) is an antibody with high affinity for the C5 component of the complement. Crovalimab inhibits the breakdown of C5 into C5a and C5b, preventing the formation of a membrane-attacking complex. Thus, crovalimab suppresses complement-dependent intravascular hemolysis in patients with nocturnal paroxysmal hemoglobinuria[91, 92].

*Tarlatamab-dlle*

Tarlatamab-dlle (IMDELLTRA™, solution for intravenous administration) is a bispecific delta-like ligand 3 (DLL3) directed to capture CD3-cells. It is intended for the treatment of advanced small cell lung cancer at the time of progression or after therapy with platinum medicines in adults. Tarlatamab-dlle causes T-cell activation, release of pro-inflammatory cytokines and lysis of cells expressing DLL3. The medicine showed antitumor activity in a mouse model of small cell lung cancer[93, 94].

## mAb of anti-inflammatory action

*Lebrikizumab-lbkz*

Lebrikizumab-lbkz (EBGLYSS, solution for subcutaneous injection) is a mAb (IgG4) that blocks IL-13, used to treat moderate to severe atopic dermatitis in children over 12 years of age and adults, with a body weight of at least 40 kg, with ineffectiveness or contraindications to the use of topical drugs. The medicine can be used in combination with topical corticosteroids. Lebrikizumab, binding to IL-13, allows it to bind to the α1 receptor to IL-13, while suppressing

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[86] ZIIHERA. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/761416s000lbl.pdf
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the signal transduction pathway through the receptor complex e IL-4Rα/IL-13Rα1. IL-13 is a cytokine involved in the development of type II inflammation, plays an important role in the pathogenesis of atopic dermatitis. By interfering with the work of IL-13, the medicine suppresses the release of pro-inflammatory cytokines, chemokines and IgE[95, 96].

*Axatilimab-csfr*

Axatilimab-csfr (NIKTIMVO™, solution for intravenous administration) is a mAb that blocks the receptor to colony-stimulating factor 1 (CSF-1R), used to treat chronic graft-versus-host disease. Blocking CSF-1R reduces the concentration of circulating pro-inflammatory and profibrotic monocytes and monocyte-derived macrophages. This effect leads to a decrease in the number of non-classical monocytes (cluster of differentiation [cluster of differentiation, CD] 14+, CD16+), which suppresses the activity of pathogenic macrophages in tissues[97, 98].

*Nemolizumab-ilto*

Nemolizumab-ilto (NEMLUVIO®, solution for subcutaneous injection) is a humanized mAb (IgG2) that selectively binds to the IL-31 receptor, intended for the treatment of nodular prurigo. IL-31 is involved in the pathogenesis of prurigo — inflammation, epithelial deregulation and fibrosis. Nemolizumab-ilto inhibits IL-31-mediated reactions, including the release of cytokines and chemokines[99, 100.]

**Others**

*Marstacimab-hncq*

Marstacimab-hncq (HYMPAVZI, solution for subcutaneous injection) is a human IgG1 mAb to the Kunitz 2 domain of tissue factor pathway inhibitor (TFPI). TFPI is an anticoagulant — the main inhibitor of coagulation activation via the extrinsic pathway. It binds to the active site of factor $X_a$ (Stuart-Prower) using the Kunitz domain. Inhibition of TFPI with marstacimab enhances coagulation, therefore it is used to reduce the frequency of bleeding episodes in adults and children over 12 years of age with hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency)[101, 102].

*Concizumab-mtci*

Concizumab-mtci (ALHEMO®, solution for subcutaneous injection) is a mAb-antagonist of TFPI, used for common prophylaxis and reduction of the frequency of bleeding episodes in adults and children over 12 years of age with hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency)[103, 104].

*Donanemab-azbt*

Donanemab-azbt (KISUNLA, solution for intravenous administration) is a humanized IgG1 mAb directed to aggregated forms of insoluble N-terminally truncated pyroglutamate beta-amyloid, intended for the treatment of Alzheimer's disease. The accumulation of amyloid plaques in the brain is a key pathophysiological mechanism in the development of Alzheimer's disease. Donanemab reduces the number of beta-amyloid plaques in the brain[105, 106].

**Imaging agents**

*Iomeprol*

Iomeprol (IOMERVUTM, solution for intra-arterial or intravenous administration) is a radiographic iodinated contrast agent used during intra-arterial procedures:

- Cerebral arteriography, including intra-arterial digital subtraction angiography (IA-DSA), in adults and children
- Visceral and peripheral arteriography and aortography, including IA-DSA, in adults and children;
- Coronary arteriography and cardiac ventriculography in adults;
- Radiographic assessment of heart chambers

[95] Drugs.com. Ebglyss. Available from: https://www.drugs.com/ebglyss.html
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and adjacent arteries in pediatric patients;
During intravenous procedures:

- CT of the head and body in adults and children;
- CT angiography of intracranial, visceral arteries and arteries of the lower extremities in adults and children;
- CT angiography of coronary vessels in adults and children;
- CT urography in adults and children.

The mechanism of action of iomeprol (Fig. 22A) is based on its ability to penetrate into the tissue of blood vessels and other structures of the body and slow down X-ray photons. Iodinated contrast agents (CA) diffuse from blood vessels into the extravascular space. In the brain with an intact BBB, CA does not diffuse into the extravascular space, and contrast enhancement is usually associated with the presence of CA in the vascular space. In patients with BBB damage, CA accumulates in the extravascular space in the area of the disorder[107, 108].

*Flurpiridaz*

Flurpiridaz F18 (FLYRCADO™, solution for intravenous administration) is a radiopharmaceutical indicated for myocardial perfusion imaging with positron emission tomography. It is used at rest or during pharmacological/physical stress on the heart in adult patients with coronary artery disease to assess the severity of ischemia and myocardial infarction. Flurpiridaz F18 (Fig. 22B) is a structural analogue of pyridabene — an inhibitor of mitochondrial complex 1. Flurpiridaz F18 is excreted from the myocardium in proportion to the blood flow rate in it and binds to active mitochondria. Thus, the detectable radioactivity in the viable myocardium is higher than in the ischemic tissue[109, 110].

*Pegulicianine*

Pegulicianine (LUMISIGHT™, solution for intravenous administration) is an imaging agent used in adult patients with breast cancer during lumpectomy as an auxiliary agent for detecting cancerous tissue in the resection cavity after removal of the primary tumor. Pegulicianine (Fig. 23) is a prodrug that does not have optical activity. When the peptide bond in the pegulicianine molecule is cleaved under the action of cathepsins and matrix metalloproteinases, “fragment 2” and “fragment 3”, which are fluorescent metabolites, are formed as a result of enzymatic cleavage. “Fragment 1” is a fluorescence quencher, its cleavage leads to the activation of molecules. Since the amount of cathepsins and metalloproteinases in tumor cells and cells adjacent to tumor cells is significantly greater than in healthy cells, this medicine visualizes areas of tissue affected by tumor growth. The absorption peak of fluorescent fragments of the pegulicianine molecule is 650 nm, and the emission peak is 675 nm[111, 112].

## DISCUSSION

### The main mechanisms of immunotherapy relevant to newly registered biologics

#### Tumor microenvironment and immune checkpoints

The tumor microenvironment (TME) is a complex and dynamic environment in which tumor cells develop. It consists of various cellular and molecular components that interact with each other and with tumor cells, forming a unique ecosystem that promotes cancer progression [7, 8].

The TME of the tumor consists of cancer cells, stromal cells (fibroblasts and others), as well as immune cells — predominantly macrophages and T-lymphocytes. The extracellular environment of the TME contains signaling ligands that bind to receptors located on the surface of tumor cells, antigen-presenting cells and immune cells. The interaction between immune cells and the tumor plays a key role in determining the dynamics of the pathological process [9–11].

Immune checkpoints are inhibitory receptors and signaling pathways that are involved in the regulation of the immune response. They play a key role in maintaining autotolerance and preventing an excessive immune response that can lead to damage to the body’s own tissues. Immunotherapy of cancers is aimed, among other things, at the interaction of the drug product with targets that are part of the system of immune checkpoints [12, 13].

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[108] IOMERVU. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/216017s000,216017s000lbl.pdf
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[111] Drugs.com. Lumisight. Available from: https://www.drugs.com/pro/lumisight.html
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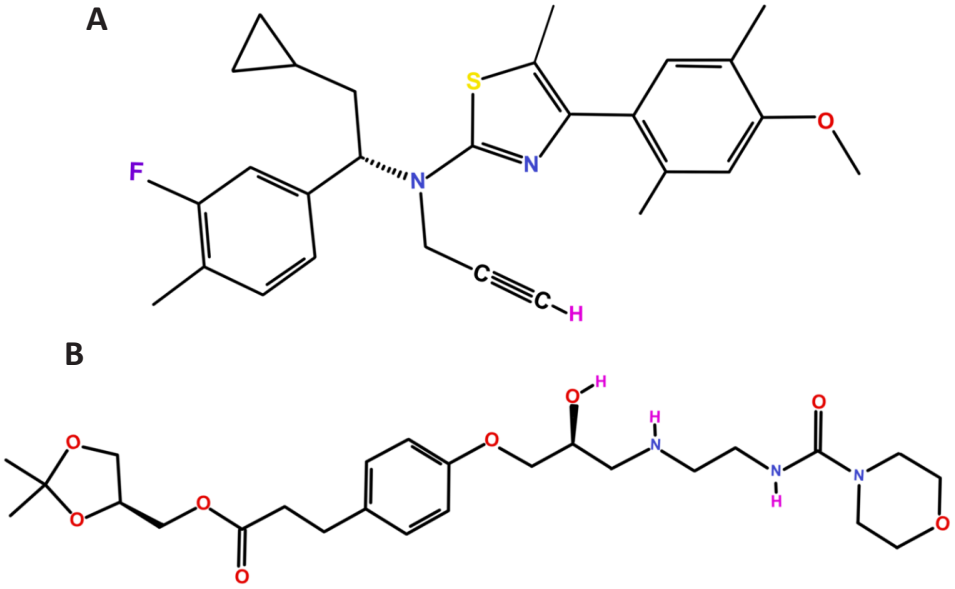


**Figure 3 – Structures of crinecerfont (A) and landiolol (B)**

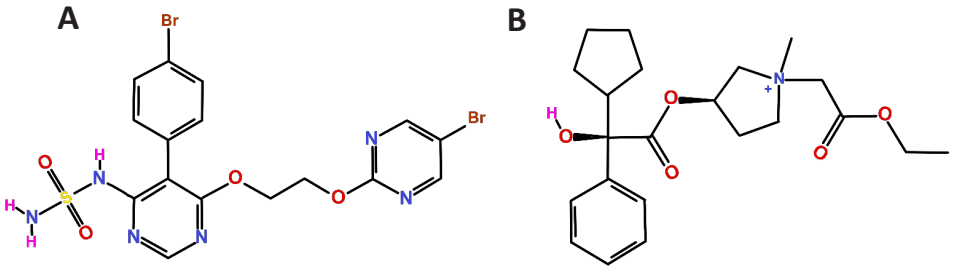


**Figure 4 – Structures of aprocitentan (A) and sofpironium (B)**

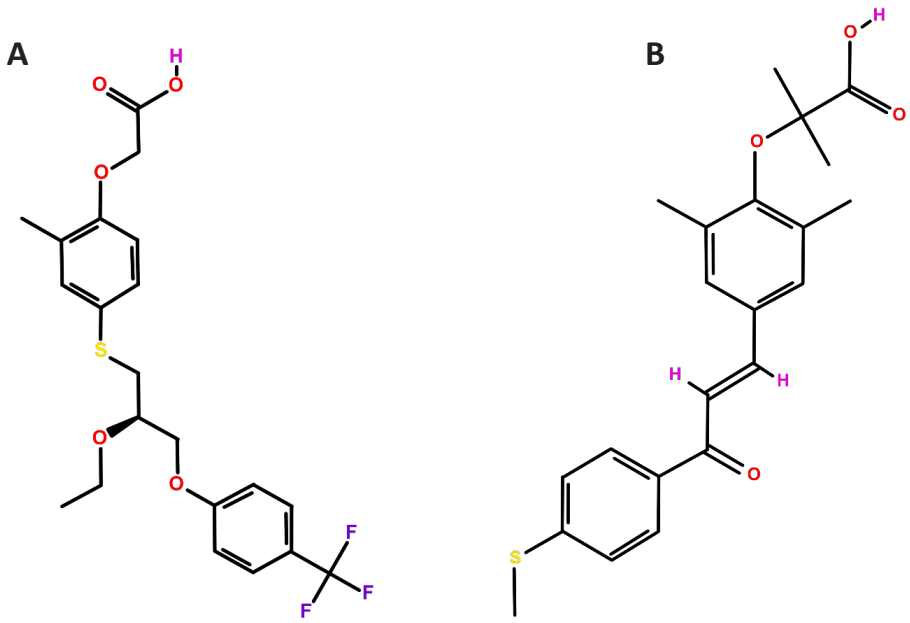


**Figure 5 – Structures of seladelpar (A) and elafibranor (B)**

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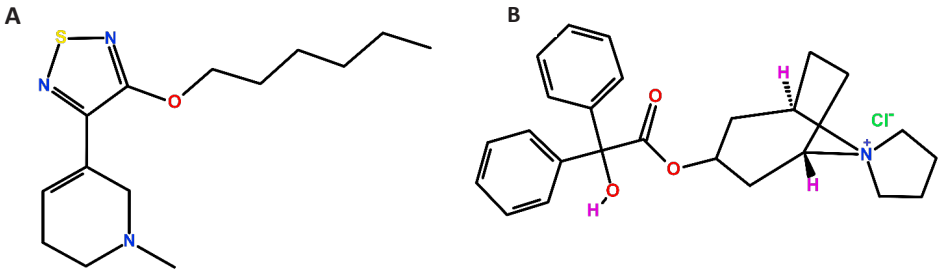


**Figure 6 – Structures of xanomeline (A) and trospium chloride (B)**

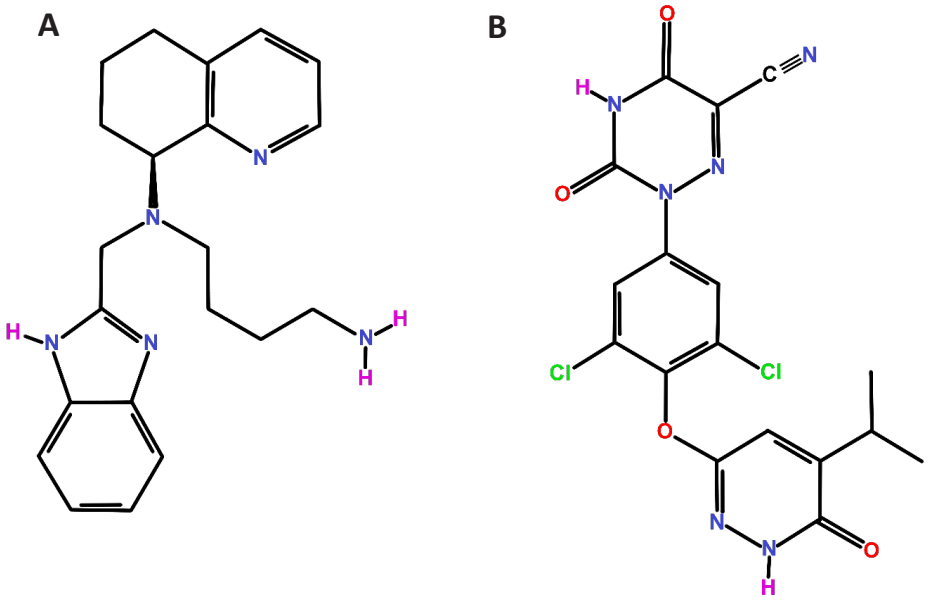


**Figure 7 –Structures of mavorixafor (A) and resmetirom (B)**

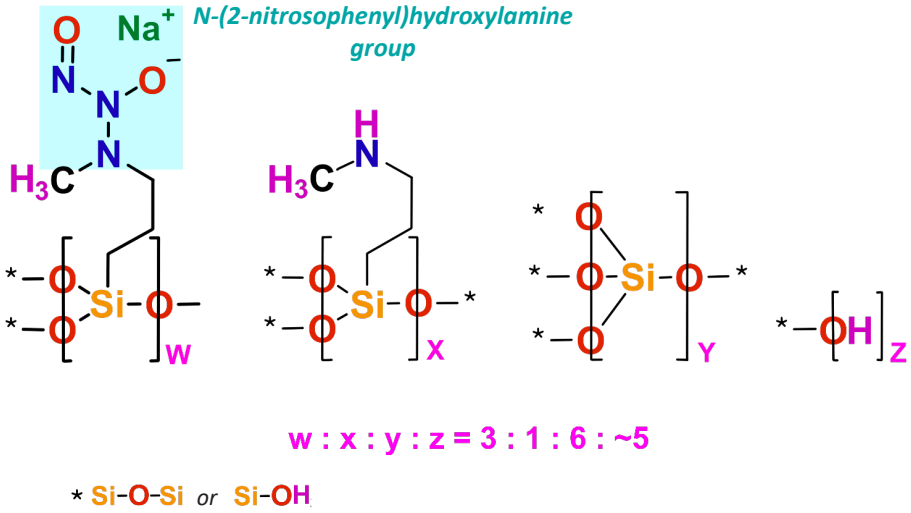


**Figure 8 – Structural formula of berdazimer**

Note: * is a common oxygen atom that binds structural components of the molecule through polyxyloxane fragments Si-O-Si or Si-OH.

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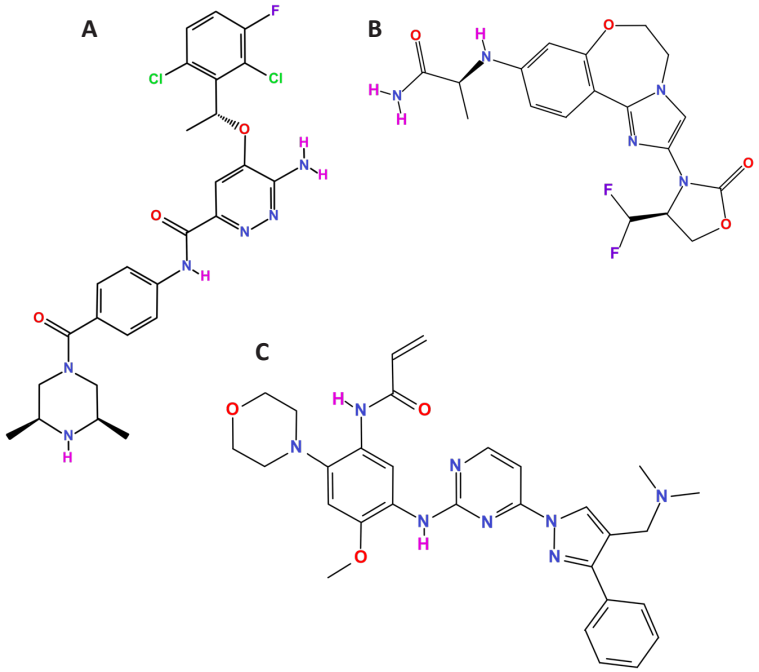


**Figure 9 – Structures of ensartinib (A), inavolisib (B) and lazertinib (C)**

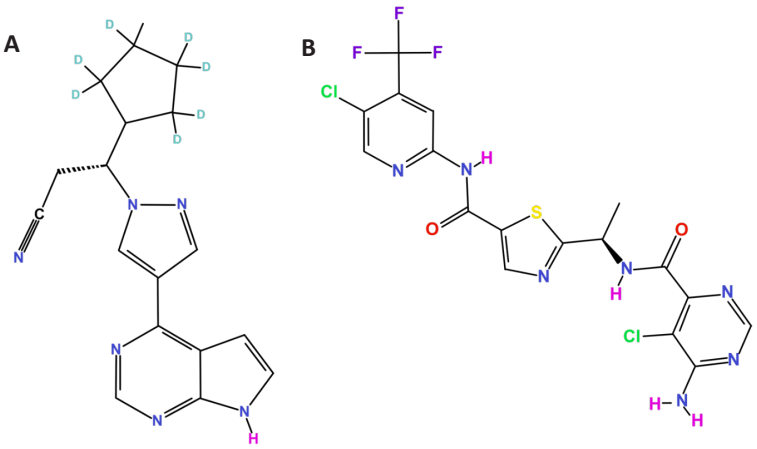


**Figure 10 –Structures of deucravacitinib (A) and ensifentrine (B)**

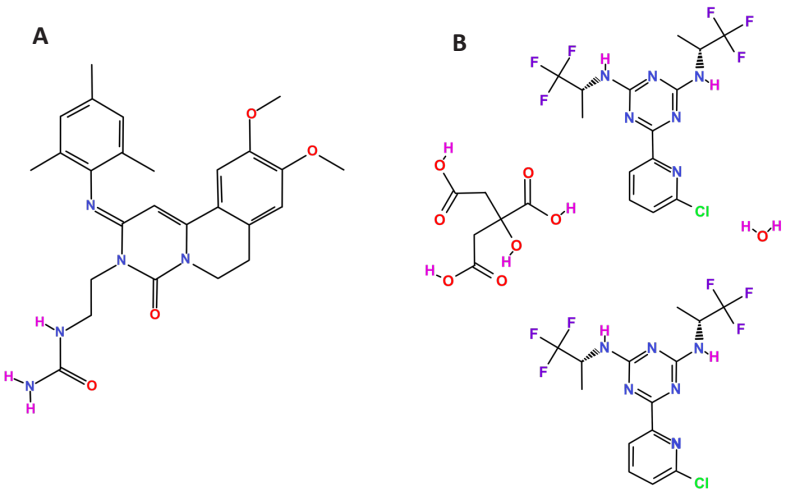


**Figure 11 – Structures of tovorafenib (A) and vorasidenib cocrystal**

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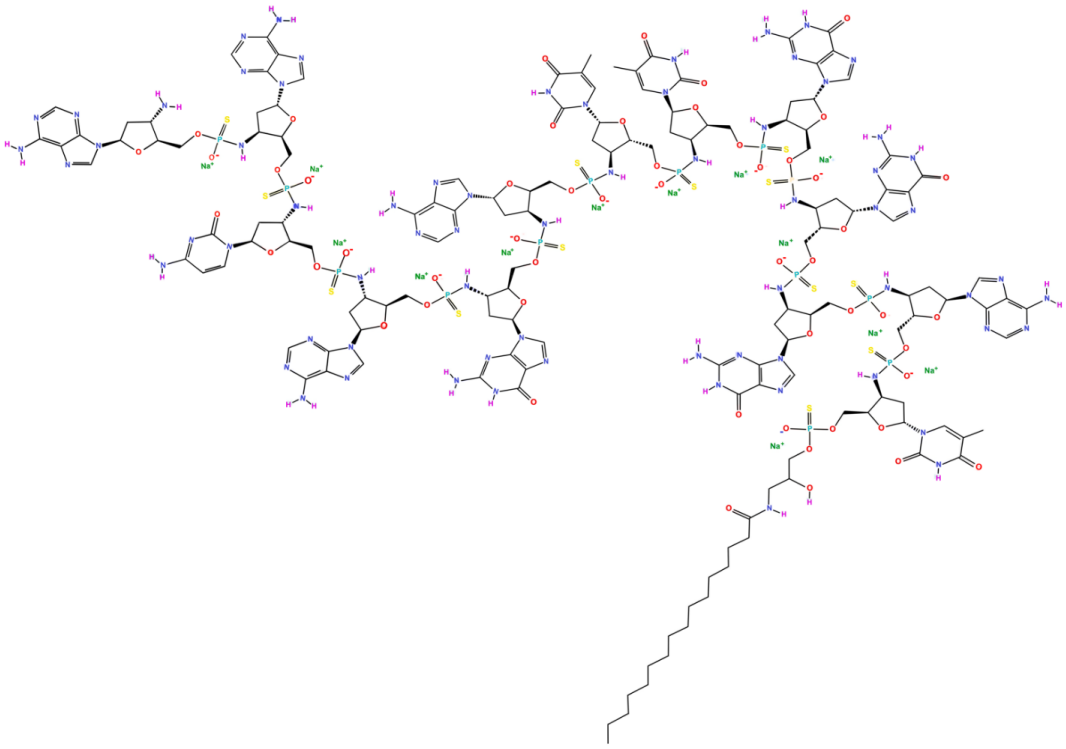

**Figure 12 – Structures of imetelstat sodium**

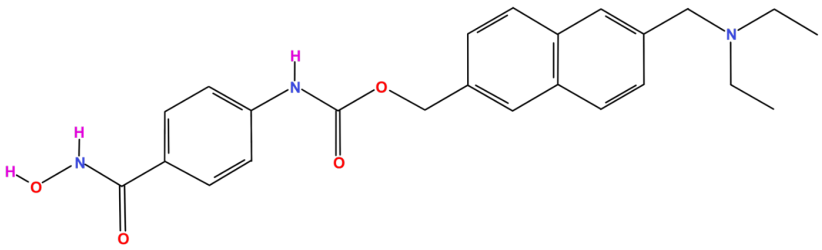

**Figure 13 – Structures of givinostat**

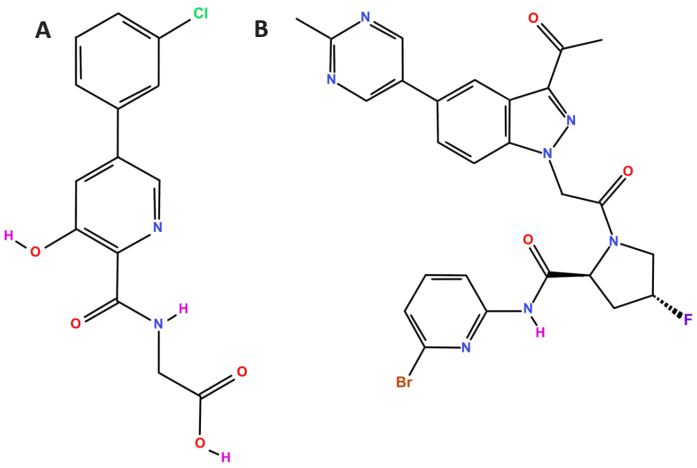


**Figure 14 – Structures of vadadustat (A) and danicopan (B)**

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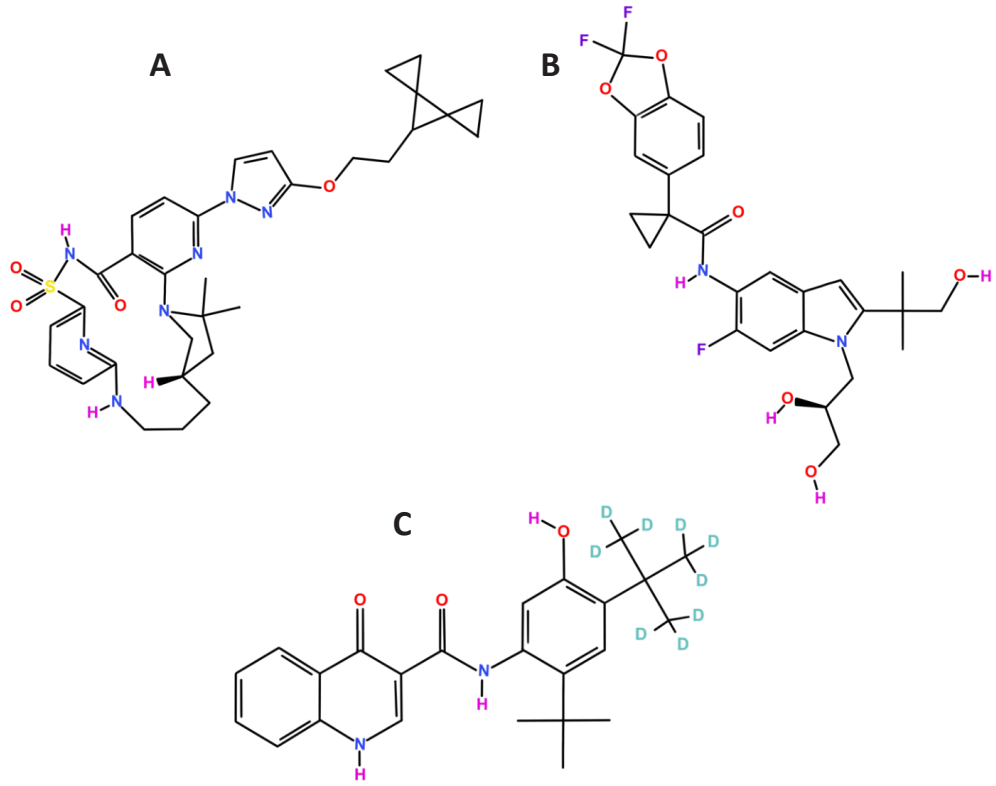


**Figure 15 – Structures of vanzacaftor (A), tezacaftor (B) and deutivacaftor (V)**

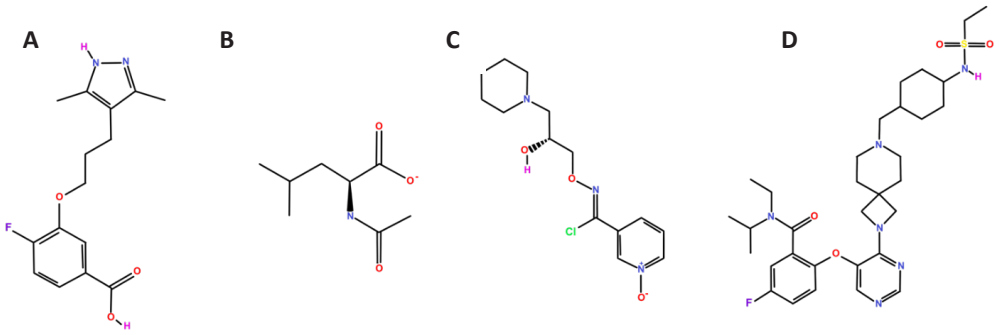


**Figure 16 – Structures of acoramidis (A), levacetylleucine (B), arimoclomol (C) and revumenib (D)**

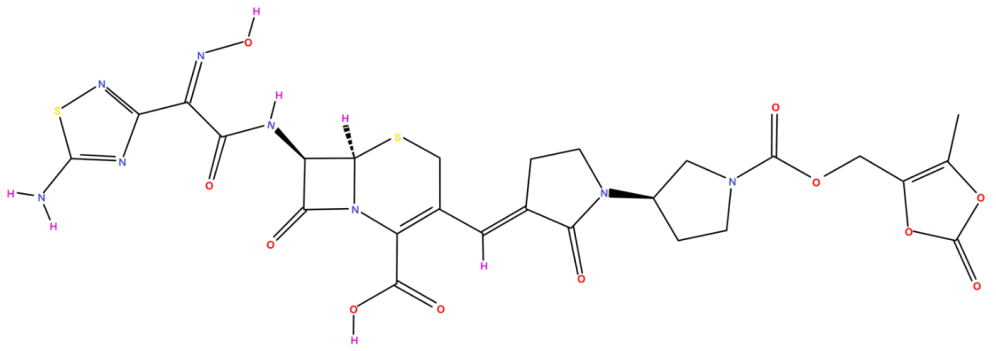

**Figure 17 – Structures of ceftobiprole medocaril**

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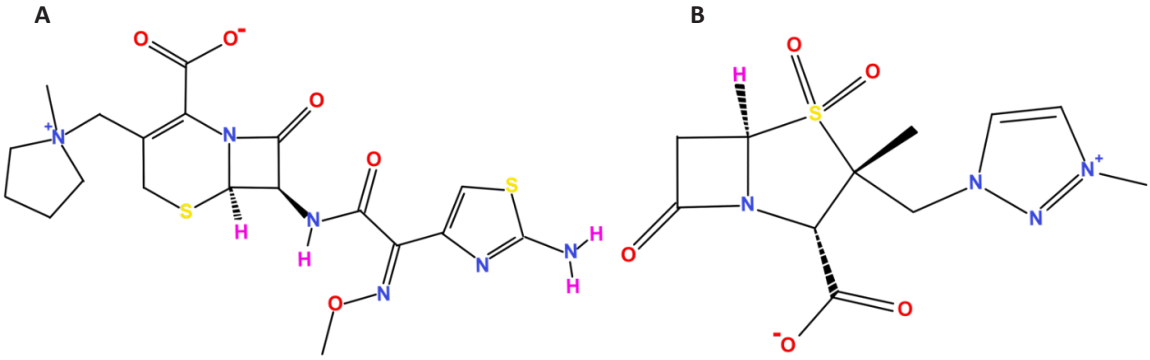


**Figure 18 – Structures of cefepime (A) and enmetazobactam (B)**

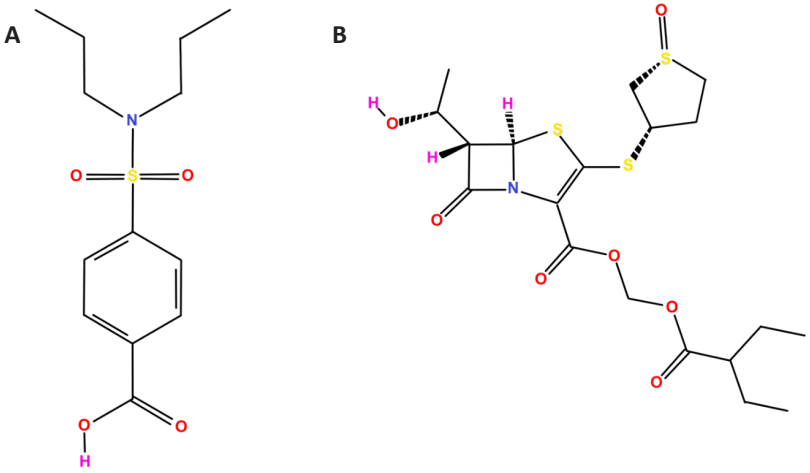


**Figure 19 – Structures of probenecid (A) and sulopenem etzadroxil (B)**

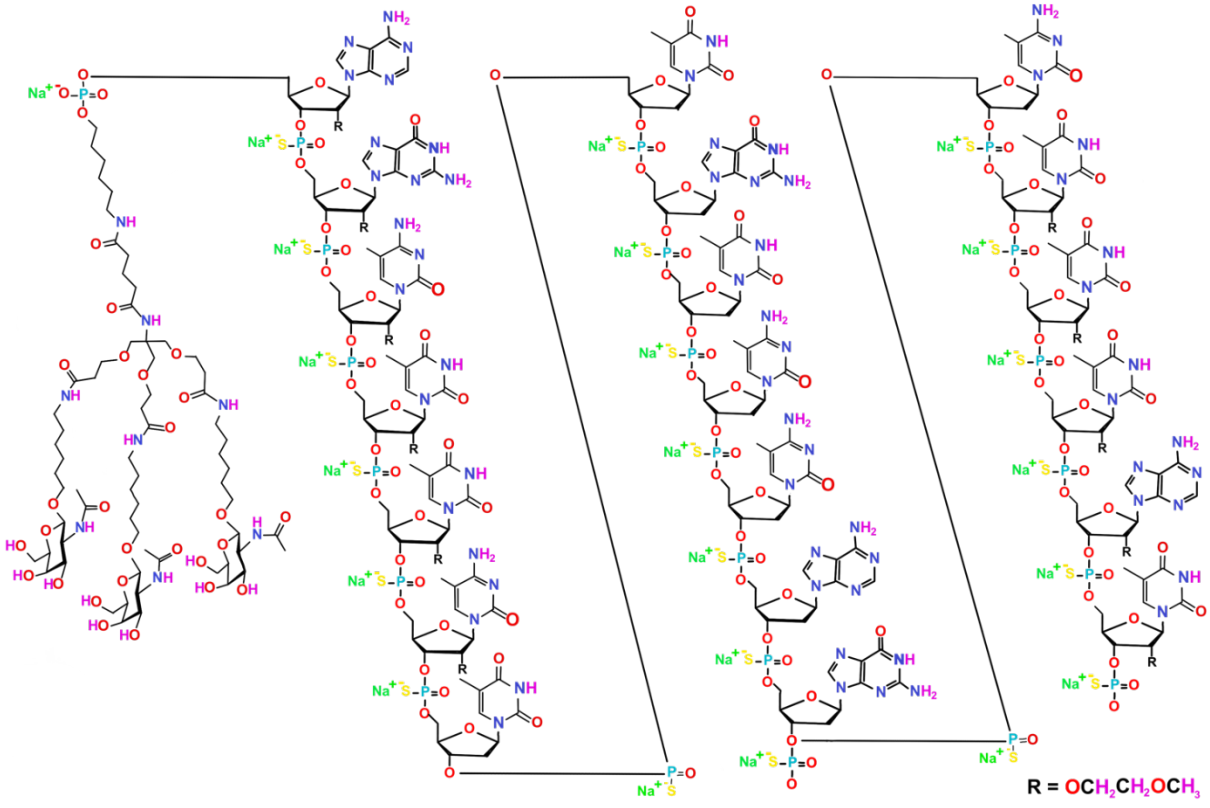


**Figure 20 – Structures of olezarsen**

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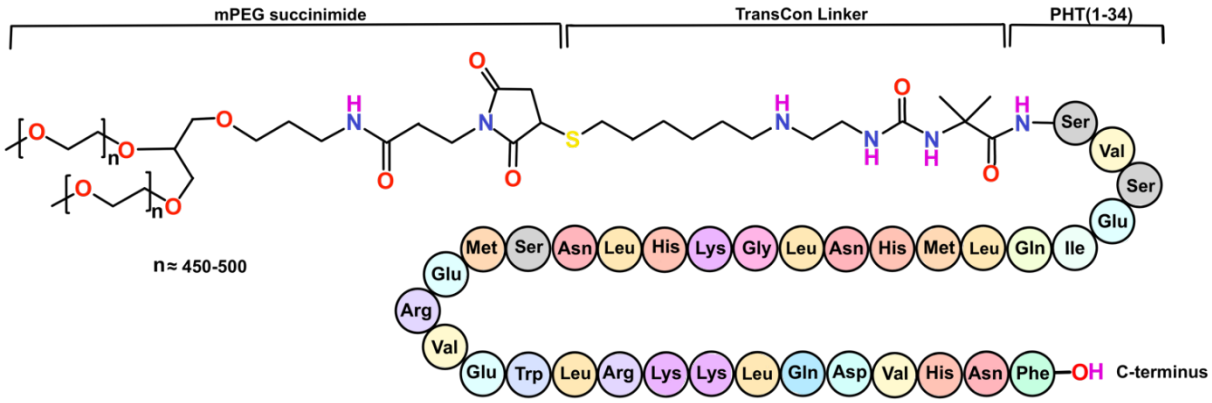


**Figure 21 – Structure of palopegteriparatide**

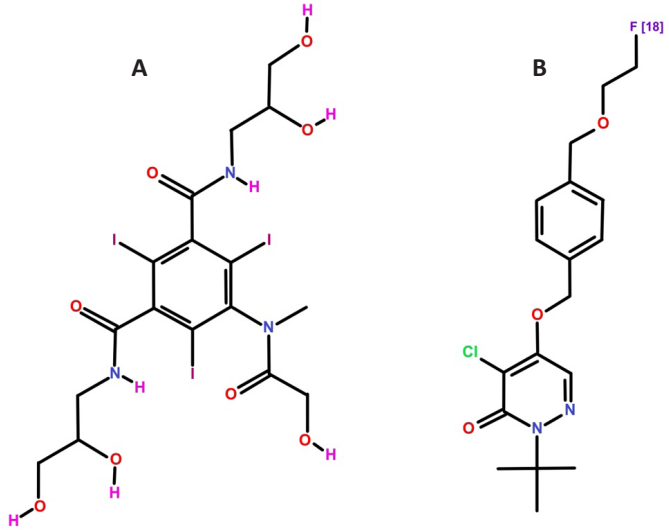


**Figure 22 – Structuresla of iomeprol (A) and flurpiridaz F18 (B)**

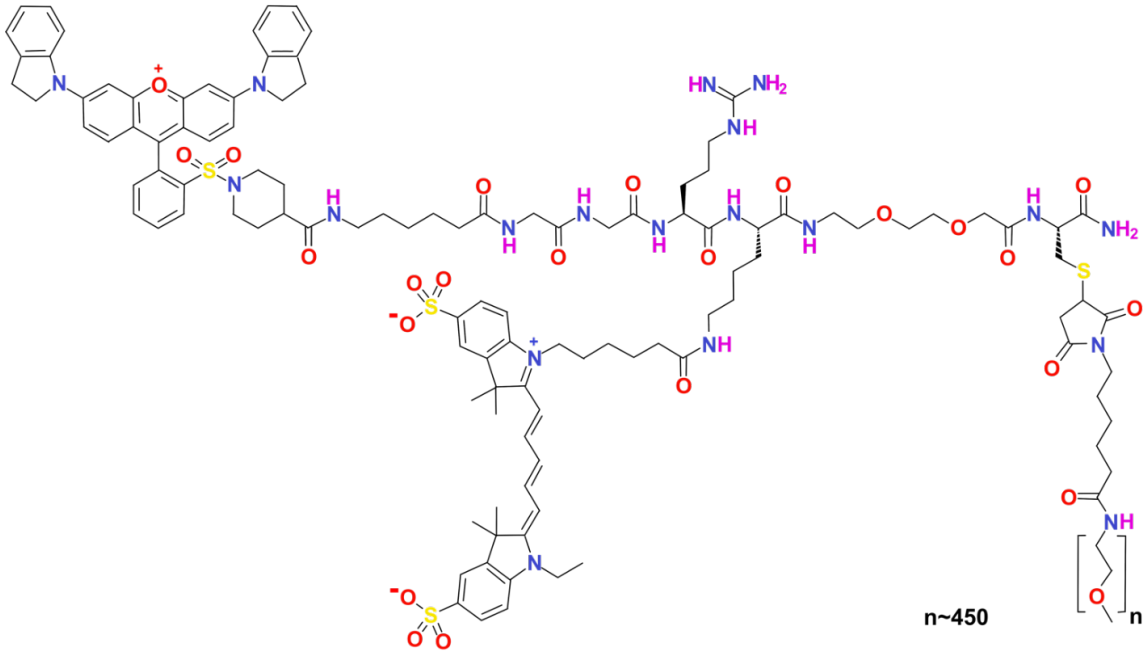


**Figure 23 – Structures of pegulicianine acetate**

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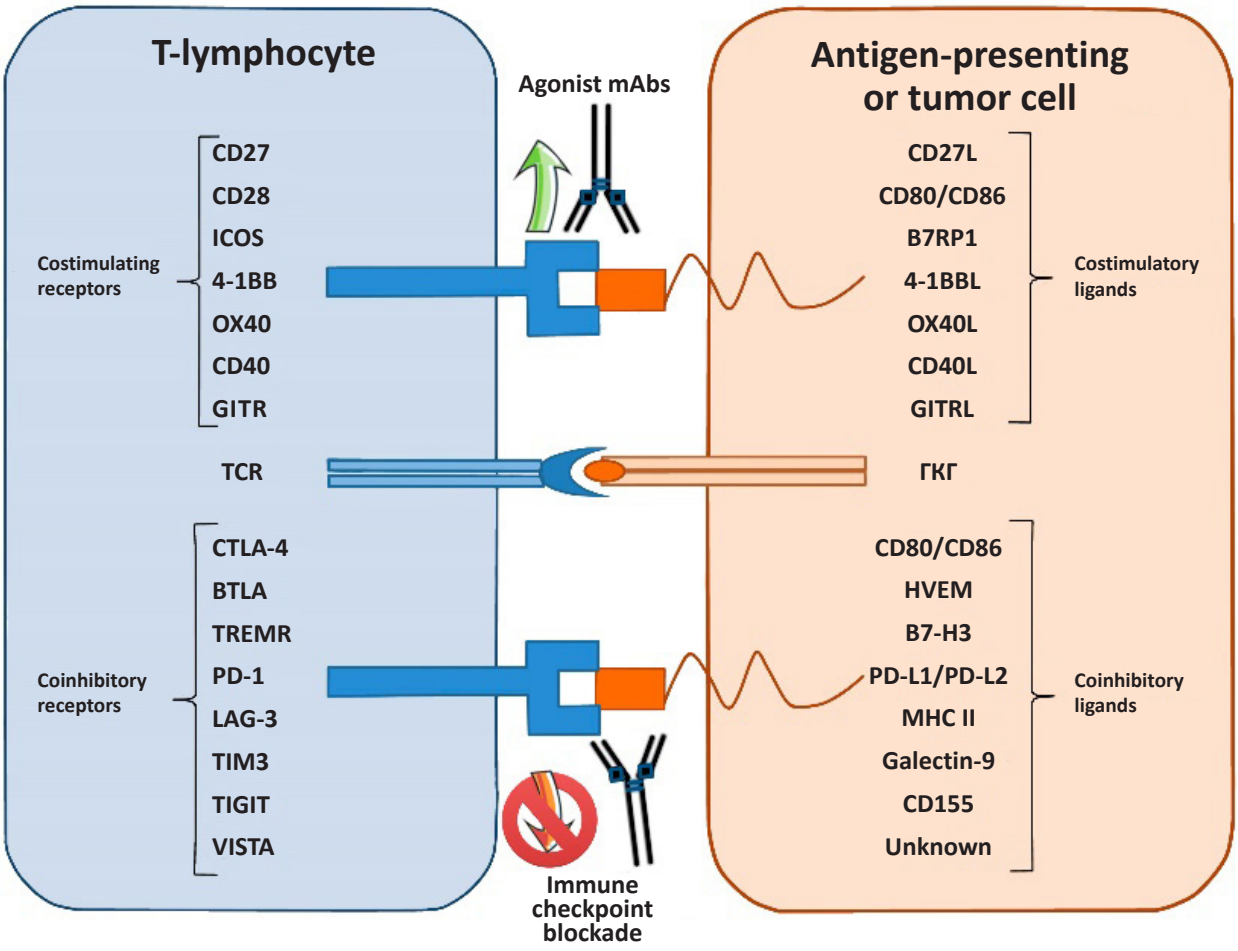


**Figure 24 – Mechanisms and factors involved in the regulation of Immune checkpoint**

Note: mAbs — monoclonal antibodies; CD — cluster of differentiation; ICOS — inducible costimulator; 4-1BB — receptor from the superfamily of tumor necrosis factor receptors, cluster of differentiation 137 (tumor necrosis factor ligand superfamily, member 9); 4-1BBL — 4-1BB ligand; OX40 — receptor from the superfamily of tumor necrosis factor receptors, cluster of differentiation 134 (tumor necrosis factor receptor superfamily, member 4); OX40L — OX40 ligand; GITR — glucocorticoid-induced TNFR-related protein; GITRL — GITR ligand; TCR — T-cell receptor; CTLA-4 — cytotoxic T-lymphocyte-associated protein 4; BTLA — B- and T-lymphocyte attenuator; TREMR — triggering receptor expressed on myeloid cells, cluster of differentiation 354; PD-1 — programmed cell death receptor 1; PD-L1/PD-L2 — ligands 1 and 2 of the programmed cell death receptor; LAG-3 — membrane immunoglobulin, gene product 3 activated by lymphocytes, cluster of differentiation 223 (lymphocyte-activation gene 3); TIM3 — T-cell immunoglobulin and mucin-domain containing-3; TIGIT — T-cell immunoreceptor with Ig and ITIM domains; VISTA — V-domain Ig suppressor of T cell activation; B7RP1 — RP1 protein of the B7 family; HVEM — Herpesvirus entry mediator, TNFRSF14; B7-H3 — H3 protein of the B7 family (cluster of differentiation 276); MHC — major histocompatibility complex.

Immune checkpoint play a key role in the activation of T-cells and determine the effects that occur when various ligands act on the T-cell receptor (TCR). Blocking the immune checkpoints CTLA-4 and PD-1 has already become one of the most successful methods of cancer immunotherapy. Promising applied points are proteins of the B7 family [14] — B7-H3 [15, 16], B7S1 [17, 18] and VISTA [19, 20].

B7-H3 can have both an inhibitory and an activating effect on T-cells. Studies show that its expression can contribute to tumor regression and increase the immunogenicity of tumors, contributing to the development of specific CD8+ cytotoxic T-cells. In people with B7-H3 deficiency, an increase in tumor size was noted [21, 22]. The role of B7-H3 is controversial, since in some cases it can act as an inhibitor of the T-cell response, depending on the expression of isoforms and the fucosylation pattern of the molecule on cells. B7-H3 also affects the migration and inhibition of cellular invasion of tumor cells, which is supposedly one of the mechanisms of its action in pancreatic cancer cells and other types of cancer. Thus, B7-H3 can act as an activator for some immunobiological cascades, and as an inhibitor for others. This protein is promising for studying its ligands for immunotherapy [14].

B7S1 is recognized as a negative regulator of T-cell responses, since its binding to receptors on T-cells leads to suppression of their proliferation, cytokine secretion and the development of effector functions.

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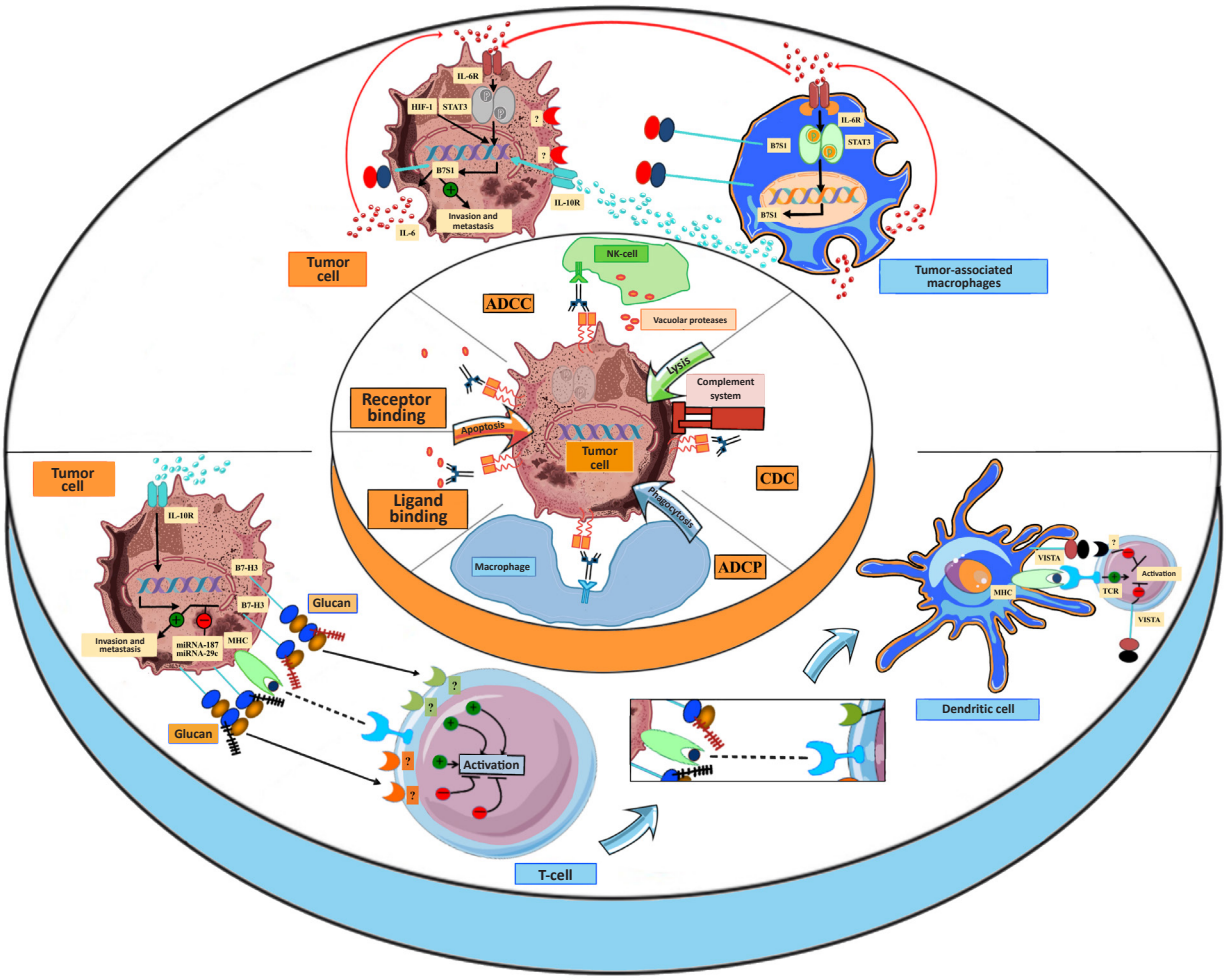


**Figure 25 – Some mechanisms involved in the antitumor action of mAbs**

Note: the symbol "?" indicates unidentified factors of the process; IL-6 – interleukin 6; IL-6R – interleukin 6 receptor; IL-10R – interleukin 10 receptor; HIF-1 – Hypoxia-inducible factor 1; STAT3 – signal transducer and activator of transcription 3; B7S1 – protein S1 of the B7 family (integral protein of antigen-presenting cells, transmitting a co-stimulatory signal to T-cells); ADCC – antibody-dependent cellular cytotoxicity; Natural Killer Cell – NK-cell (natural killer); CDC – complement-dependent cytotoxicity; ADCP – antibody-dependent cellular phagocytosis; B7-H3 – protein H3 of the B7 family (cluster of differentiation 276); MHC – major histocompatibility complex; miRNA-187 – microRNA 187 product; miRNA-29c – microRNA 29c product; TCR – T-cell receptor; VISTA – V-domain Ig suppressor of T cell activation.

"B7S1 contributes to protecting tumor cells from the anti-tumor immune response. Transformation of T cells under the influence of B7S1 led to their arrest in the cell cycle and an increase in apoptosis levels. B7S1 may support tumor growth by promoting immunosuppression in the tumor microenvironment. Binding of B7S1 to receptors disrupts the phosphorylation of key kinases such as ERK and AKT, which in turn reduces T- cell proliferation and IL-2 secretion. B7S1 may facilitate the metastasis of cancer cells by enabling them to evade the immune response [14]."

VISTA functions as a negative regulator of T-cell activation. It suppresses early T-cell activation, preventing their proliferation and secretion of cytokines such as interferon (IFN) γ and TNF-α. Thus, B7S1 and VISTA are co-inhibitors that suppress T-cell activation at different stages of this process. VISTA has an established role in maintaining T-cells in a state of tolerance through mechanisms aimed at weakening T-cell activity when interacting with APCs [14].

The main receptor of T-lymphocytes is the CD28 molecule, which is present on all naive T-leukocytes. Ligands for CD28 on the surface of antigen-presenting cells (APCs) are B7.1 (CD80) and B7.2 (CD86) molecules. The interaction of CD28 with these ligands leads to the activation of phospholipase C, Akt, and Vav

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enzymes, which enhances most of the effects caused by TCR stimulation. These processes are only possible with the simultaneous arrival of two signals. Receptors of the TNF family (OX40, 4-1BB, CD30, and CD27) are the main co-stimulatory receptors of B-lymphocytes and activate Akt and NFκB. In addition, stimulation can occur through direct interaction of the pathogen with pattern recognition receptors, such as Toll-like receptors (TLRs). Currently, the concept of co-stimulation is being revised and expanded due to the discovery of new co-stimulatory receptors that implement their functions through various mechanisms. It has been demonstrated that co-stimulation, for example, through the GITR receptor, not only enhances TCR signaling but also participates in determining the direction of T-cell differentiation [23]. Inhibitors of co-stimulatory receptors may become the basis for the development of new safe and effective treatments for graft-versus-host disease [24].

### Hybrid proteins

Hybrid proteins (fusion proteins) are molecules that are formed as a result of the combination of two or more genes that are initially located in different regions of the genome [25]. This fusion leads to the formation of a new protein that may have unique properties different from the original proteins. Hybrid proteins are involved in a number of biological processes, such as the translation of genetic information and cell signaling pathways [26].

The merging process can occur through various mechanisms, including errors in DNA replication, gene recombination, or chromosomal translocations [27]. Often, such changes may be associated with the development of diseases, including cancer.

Hybrid proteins are considered an important marker of malignancy, as they arise from genetic changes that can cause uncontrolled cell growth. Examples include the transformation of cells into cancerous ones through the activation of oncogenes, such as BCR-ABL1, which is a hybrid protein typical for chronic myelogenous leukemia [28].

Gene fusion can lead to the expression of proteins with increased enzymatic activity or proteins that regulate key cellular processes, such as the cell cycle, apoptosis, or signaling pathways, leading to uncontrolled cell division and tumor development [29].

Currently, it is known if hybrid proteins can be the result of random mutations. The detection of hybrid proteins in tumor cells is rarely used as a marker for monitoring the course of the disease and personalizing treatment. Hybrid proteins can be targets for targeted therapy, such as tyrosine kinase inhibitors, for the treatment of chronic myelogenous leukemia [30, 31].

### Monoclonal antibodies and their mechanisms of action

Among the 16 registered biologics, 12 are immunotherapeutic. Thus, among the medicines registered with the FDA in 2024, 24% are mAbs. In 2023, 12 antibodies and 1 antibody-protein conjugate medicine were registered.

The most commonly used group in mAb therapy is IgG, since this class of antibodies interacts with the type of FcR, FcγR, associated with them, found on NK, as well as neutrophils, monocytes, dendritic cells, and eosinophils to participate in the performance of specialized functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The IgG class can be divided into groups depending on the ability of the Fc region to perform these functions. IgG1 and IgG3 are capable of causing ADCC and CDC, while IgG2 and IgG4, on the contrary, cannot [32]. IgG1 is the most relevant subclass of monoclonal antibodies used in cancer immunotherapy [33].

Previously, the mechanism of action of mAbs with antitumor effects was justified by the action on a receptor or other molecule expressed on the surface of a tumor cell. Over the past few years, it has been found that the action of mAbs is multifactorial — a large role is now assigned to their regulatory properties. Recently, the most successful mAb-based strategies have moved away from targeting tumor antigens and focused on interacting with immune cells to enhance their antitumor potential. One of the first approaches to stimulating antitumor immunity with mAbs was the development of bispecific T-cell engagers (BiTEs), which simultaneously target a tumor antigen, such as CD19, and the activating receptor CD3 on T-cells. BiTEs combine direct effects on tumor cells with the recruitment of cytotoxic T-cells into the TME of the tumor and lead to tumor regression even when administered at doses three orders of magnitude lower than the parent mAb alone. One of the first approaches to stimulating antitumor immunity with mAbs was the development of bispecific antibodies BiTEs, which simultaneously target a tumor antigen, such as CD19, and the activating receptor CD3 on T-cells. BiTEs combine direct effects on tumor cells with the recruitment of cytotoxic T-cells into the TME of the tumor and lead to tumor regression even when administered at doses significantly lower than the parent mAbs alone [34].

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Targeted mAbs, by binding to antigens unique to tumor cells or expressing antigens excessively, can cause tumor cell death through various mechanisms. The main direct mechanism causing tumor cell death is blocking the signal from growth factor receptors (Fig. 25). Signaling that promotes tumor growth and survival is disrupted when mAbs bind to target growth factor receptors and alter their activation state or block ligand binding. For example, EGFR expression is elevated in many cancers, and signaling through EGFR leads to proliferation, migration, and invasion of tumor cells. KG anti-EGFR mAbs cause apoptosis in tumor cells by blocking ligand binding and receptor dimerization [34, 36].

ADCC is an immune mechanism that increases the specificity of immunity against cancer and infected cells and the ability to destroy them. ADCC is an immune response mediated primarily by NK cells, which are a type of lymphocyte. ADCC plays a key role in cancer immunotherapy when using mAbs. ADCC develops with the participation of a large number of effectors, primarily with the participation of NK cells. However, the mechanism affects other cells of the myeloid series — monocytes, macrophages, neutrophils, eosinophils, and dendritic cells [37].

Antibodies act as bridges, linking antigens on the surface of tumor cells through their Fab portions and effector cells through Fc fragments. For ADCC to occur, effector cells must express Fc receptors (FcR) that bind to antibodies. The main class of FcR associated with ADCC is FcγR, which includes activating receptors such as FcγRI (CD64), FcγRIIA (CD32A), and FcγRIIIA (CD16A), as well as inhibitory FcγRIIB (CD32B). Effector cells cause the death of target cells through the release of cytotoxic granules, Fas signals, and the initiation of reactive oxygen species. The effectiveness of many targeted mAbs in clinical practice largely depends on ADCC. Some mechanisms of resistance to therapy may be associated with depletion of NK cells and their reduced cytotoxic activity [37].

Most targeted mAbs are capable of activating the complement system. For example, the effectiveness of rituximab in vivo partially depends on CDC. In a preclinical model, the antitumor effects of rituximab were investigated in animals with a knockout of the C1q complement cascade component gene. In such animals, a complete absence of the effectiveness of the studied medicine was revealed [38]. The importance of CDC in mAb therapy is further confirmed by the fact that genetic polymorphisms in the C1qA gene correlate with the clinical response to rituximab in patients with follicular lymphoma [39].

ADCP studies are very limited, but there is some evidence that ADCP plays an important role in the destruction of circulating tumor cells after mAb therapy [40].

Each class of antibodies has a corresponding class of FcR, for example, FcγR, which binds IgG, and FcαR, which binds IgA. FcγR is the most significant class for ADCC of tumor cells and includes both activating FcγRI (CD64), FcγRIIA (CD32A), FcγRIIIA (CD16A), and inhibitory FcγRIIB (CD32B) receptors [41]. In additional studies to elucidate the mechanism of action using similar mouse models, it was confirmed that the expression of FcγR by immune effector cells is necessary for tumors to respond to mAb therapy [42]. When the activating FcγR on the effector cell binds the Fc region of the antibody receptor, a signal is propagated downstream. NK cells are the main type of effector cells that mediate ADCC; however, other cells of the myeloid series, such as monocytes, macrophages, neutrophils, eosinophils, and dendritic cells, are also capable of this [43].

Although many mAbs are capable of exerting effects through several of the above mechanisms, there is debate if they are important in vivo. It is known that many of the first mAb medicines mediate ADCC of tumor cells in vitro, but the question of how important ADCC is for their therapeutic effectiveness was initially little studied. Using mouse models, R.A. Clynes et al. were the first to demonstrate that ADCC is a key mechanism of action mediating the activity of trastuzumab and rituximab in vivo [44]. ADCC is the main therapeutic mechanism of rituximab in non-Hodgkin's lymphoma and anti-CD38 antibodies in multiple myeloma [45, 46].

The functionality of antibodies with respect to ADCC can be increased by modifying the Fc portion of the mAb to increase their binding affinity to the activating FcγRIIIA through site-directed mutagenesis, changing the glycosylation of the Fc domain, and/or removing the fucosylation of the Fc domain [47–50].

Rituximab — the first antibody, the drug of which was approved for the treatment of cancer — is a mAb to CD20 [51]. CD20 is a membrane protein of B-lymphocytes, the increased expression of which is a characteristic phenomenon for B-cell lymphomas. Since the registration of rituximab, the development of antitumor mAbs directed against membrane proteins of immune cells, the increased expression of which is specific and depends on the type of cancer, has intensified. Today, mAbs directed against targets such as EGFR and HER2 are widely used in the clinic for the treatment of colorectal cancer and breast cancer, respectively [52, 53].

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The tumor microenvironment contains many factors that are known to suppress the antitumor immune response, promote the growth of tumor cells, and prevent tumor angiogenesis. Targeting these crucial pro-tumor processes in the TME of the tumor has proven its clinical effectiveness. Historically, the most relevant target was VEGF, which is abundantly present in the TME of many solid tumors and binds to its receptor VEGFR, located on the endothelium of blood vessels adjacent to the tumor, stimulating angiogenesis. The inhibitor of tumor-associated macrophages, bevacizumab, targets VEGF and blocks the binding of VEGF to the receptor, is approved for the treatment of many types of cancer [54].

Currently, there are many other ways to use mAbs in cancer therapy, including antibody-drug conjugates, targeted antitumor compounds in the microenvironment, BiTEs, and immunological checkpoint inhibitors. It is possible to combine antibodies with effectors, for example, cytotoxic substances or radiopharmaceuticals. Immune checkpoints are pathways and a network of their receptors that are responsible for the homeostasis of the immune system, autotolerance, and also modulate immune reactions to limit concomitant tissue damage [55]. Such representatives of the immunoglobulin superfamily as lymphocyte activation gene 3 (LAG3), T-cell immunoglobulin and mucin domain 3 (TIM3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and V-domain Ig suppressor of T-cell activation (VISTA) are being studied as potential therapeutic targets of immunological checkpoints [14, 56].

### Registration trends

In 2024, CDER registered 26 (52%) medicines for the treatment of orphan diseases (not all of these medicines contain an orphan disease as an indication). Among the diseases that are an indication for the use of registered medicines are: Niemann-Pick disease type C, Duchenne muscular dystrophy, primary biliary cholangitis, familial chylomicronemia, classical congenital adrenal hyperplasia. Drugs have also been registered for the treatment of rare types of cancer: previously treated, unresectable or metastatic bile duct tumor positive for HER2 mutation (IHC 3+); diffuse forms of grade 2 astrocytoma or oligodendroglioma; locally advanced unresectable or metastatic HER2-negative CLDN18.2-positive adenocarcinoma of the stomach or gastroesophageal junction.

Defining a medicine as a breakthrough therapy includes all the characteristics of the Fast Track program and involves methodological support from the FDA in the medicine development process. Among the 50 registered medicines, 24 (48%) are first-in-class, and 18 of the 50 new ones (36%) are designated as breakthrough therapy. The described data are presented in Table 4.

### Drugs with new indications for use

The presented list of medicines is not included in the list of registered for the first time. Nevertheless, it should be noted that adding a new indication is an actual registration strategy.

Alectinib (Alecensa) in capsules was first approved in 2015 for the treatment of ALK-positive metastatic NSCLC in adults with progression after the use of crizotinib or with its intolerance. In 2024, Alecensa was approved as adjuvant therapy (auxiliary treatment after the main one) in adults after tumor resection in ALK-NSCLC. ALK-NSCLC is caused by a gene fusion (connection of two genes), which leads to the formation of an abnormal ALK protein that causes the growth and spread of cancer cells in the lungs[113].

Belimumab (Benlysta) for intravenous administration was originally approved in 2019 for the treatment of children aged 5 years and older with active, autoantibody-positive systemic lupus erythematosus receiving standard therapy. In 2024, Benlysta was approved in the form of a syringe pen for subcutaneous administration to children from 5 years and older, which allows them to receive treatment at home[114].

Daratumumab+hyaluronidase-fihj (Darzalex Faspro) for subcutaneous administration was originally approved in 2020 for the treatment of multiple myeloma. In 2024, CDER approved Darzalex Faspro in combination with bortezomib, lenalidomide, and dexamethasone for induction and consolidation therapy in patients with newly diagnosed multiple myeloma who are candidates for autologous stem cell transplantation[115].

Fam-trastuzumab deruxtecan-nxki (Enhertu) for intravenous administration was first approved in 2019 for the treatment of unresectable or metastatic HER2-positive breast cancer. In 2024, CDER approved Enhertu for the treatment of adults with unresectable or metastatic HER2-positive (IHC 3+) solid tumors. Treatment with Enhertu is aimed at patients who have received systemic treatment and do not have satisfactory alternative treatment options.

[113] Drugs.com. Alecensa. Available from: https://www.drugs.com/alecensa.html
[114] Drugs.com. Benlysta. Available from: https://www.drugs.com/benlysta.html
[115] Drugs.com. Darzalex Faspro. Available from: https://www.drugs.com/darzalex-faspro.html

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**Table 4 – Trends in FDA drug registration**

| Drug | New registration | New indication | Drugs for the treatment of orphan diseases | First in class | Breakthrough |
|---|---|---|---|---|---|
| Alhemo | Yes | No | Yes | No | No |
| Alyftrek | Yes | No | Yes | No | No |
| Anktiva | Yes | No | No | Yes | Yes |
| Aqneursa | Yes | No | Yes | Yes | No |
| Attruby | Yes | No | Yes | No | No |
| Bizengri | Yes | No | Yes | Yes | Yes |
| Cobenfy | Yes | No | No | Yes | No |
| Crenessity | Yes | No | Yes | Yes | Yes |
| Duvyzat | Yes | No | Yes | Yes | No |
| Ebglyss | Yes | No | No | No | No |
| Ensacove | Yes | No | No | No | No |
| Exblifep | Yes | No | No | No | No |
| Flyrcado | Yes | No | No | No | No |
| Hympavzi | Yes | No | Yes | Yes | No |
| Imdelltra | Yes | No | Yes | Yes | Yes |
| Iomervu | Yes | No | No | No | No |
| Iqirvo | Yes | No | Yes | Yes | Yes |
| Itovebi | Yes | No | No | No | Yes |
| Kisunla | Yes | No | No | No | Yes |
| Lazcluze | Yes | No | No | No | No |
| Leqselvi | Yes | No | No | No | No |
| Letybo | Yes | No | No | No | No |
| Livdelzi | Yes | No | Yes | No | Yes |
| Lumisight | Yes | No | No | Yes | No |
| Miplyffa | Yes | No | Yes | Yes | Yes |
| Nemluvio | Yes | No | No | Yes | Yes |
| Niktimvo | Yes | No | Yes | Yes | No |
| Ohtuvayre | Yes | No | No | No | No |
| Ojemda | Yes | No | Yes | No | Yes |
| Orlynvah | Yes | No | No | No | No |
| Piasky | Yes | No | Yes | No | No |
| Rapiblyk | Yes | No | No | No | No |
| Revuforj | Yes | No | Yes | Yes | Yes |
| Rezdiffra | Yes | No | No | Yes | Yes |
| Rytelo | Yes | No | Yes | Yes | – |
| Sofdra | Yes | No | No | No | No |
| Tevimbra | Yes | No | Yes | No | No |
| Tryngolza | Yes | No | Yes | Yes | Yes |
| Tryvio | Yes | No | No | Yes | – |
| Unloxcyt | Yes | No | No | No | No |
| Vafseo | Yes | No | No | No | No |
| Voranigo | Yes | No | Yes | No | Yes |
| Voydeya | Yes | No | Yes | Yes | Yes |
| Vyloy | Yes | No | Yes | Yes | No |
| Winrevair | Yes | No | Yes | Yes | Yes |
| Xolremdi | Yes | No | Yes | Yes | No |
| Yorvipath | Yes | No | Yes | No | No |
| Zelsuvmi | Yes | No | No | Yes | No |
| Zevtera | Yes | No | No | No | No |
| Ziihera | Yes | No | Yes | Yes | Yes |
| Alecensa | No | Yes | Yes | No | No |
| Benlysta | No | Yes | No | Yes | No |
| Darzalex Faspro | No | Yes | Yes | Yes | Yes |
| Enhertu | No | Yes | Yes | Yes | Yes |
| Epkinly | No | Yes | No | No | No |
| Fabhalta | No | Yes | No | No | No |
| Imfinzi | No | Yes | No | No | No |
| Livmarli | No | Yes | No | No | No |
| Otezla | No | Yes | No | Yes | No |
| Rybrevant | No | Yes | Yes | Yes | Yes |
| Wegovy | No | Yes | No | No | No |
| Xolair | No | Yes | No | Yes | No |
| Zepbound | No | Yes | No | No | No |

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HER2-positive solid tumors are characterized by a high level of HER2 protein[116].

Epcoritamab-bysp (Epkinly) for subcutaneous administration was originally approved in 2023 for the treatment of relapsed or refractory diffuse large B-cell lymphoma. In 2024, CDER approved Epkinly for the treatment of adults with relapsed or refractory follicular lymphoma after two or more lines of systemic therapy[117].

Iptacopan (Fabhalta) in capsules was first approved in 2023 for the treatment of paroxysmal nocturnal hemoglobinuria. In 2024, CDER approved Fabhalta to reduce proteinuria (protein in the urine) in adults with primary immunoglobulin A (IgA) nephritis at risk of rapid disease progression[118].

Durvalumab (Imfinzi) for intravenous administration was originally approved in 2017 for the treatment of locally advanced or metastatic urothelial cancer. In 2024, CDER approved Imfinzi for the treatment of patients with resectable NSCLC without known mutations in the epidermal growth factor receptor or rearrangements of anaplastic lymphoma kinase (ALK)[119].

Maralixibat (Livmarli) in the form of an oral solution was first approved in 2021 for the treatment of cholestatic itching in patients with Alagille syndrome. In 2024, CDER approved Livmarli for the treatment of progressive familial intrahepatic cholestasis — a rare genetic disorder that prevents normal bile secretion by the liver, leading to liver disease and subsequently to liver failure[120].

Apremilast (Otezla) in tablets was originally approved in 2014 for the treatment of active psoriatic arthritis. In 2024, CDER approved Otezla for the treatment of moderate to severe plaque psoriasis in adults[121].

Amivantamab-vmjw (Rybrevant) solution for intravenous administration was first approved in 2021. In 2024, CDER approved Rybrevant as a first-line therapy for adults with locally advanced or metastatic NSCLC with EGFR exon 20 insertion mutations identified by FDA-approved tests (Guardant360 CDx, Oncomine Dx Target Test). EGFR exon 20 insertion mutations can cause uncontrolled cell growth and are a biomarker for lung cancer[122].

Semaglutide (Wegovy) solution for subcutaneous administration was originally approved in 2021. In 2024, CDER approved Wegovy to reduce the risk of serious adverse cardiovascular events (cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke) in adults with established cardiovascular disease and obesity or overweight[123].

Omalizumab (Xolair) solution for subcutaneous administration was originally approved in 2003 for the treatment of adults and adolescents (12 years and older) with moderate to severe persistent asthma. In 2024, CDER approved Xolair for the treatment of IgE-mediated food allergy in adults and pediatric patients aged 1 year and older to reduce allergic reactions (type I), including anaphylaxis, that may occur with accidental exposure to one or more foods (used in combination with allergen avoidance)[124].

Tirzepatide (Zepbound) for subcutaneous administration was first approved in 2023 for the treatment of type 2 diabetes and weight loss. In 2024, CDER approved Zepbound for the treatment of obstructive sleep apnea[125].

## CONCLUSION

In the presented work, we tried to characterize the latest achievements and trends that can be observed in the global pharmaceutical market. It is possible to identify general patterns, such as the continuing trend towards the development of ligands to receptors (most medicines belong to this group) and the desire to develop biologics, the group of which is becoming more and more large-scale and heterogeneous every year, as well as medicines for the treatment of rare diseases. This not only allows therapy for patients but also gives rapid development, gives impetus (financial, marketing, population, etc.) to the area of knowledge and technologies, resources previously unavailable or the significance considered worthy of attention. Medicines from the group of first-in-class or recognized as breakthrough technologies also demonstrate increasing human capabilities, and their presence feeds hope for a further increase in life expectancy and its quality.

According to the results of FDA approvals in 2024,

[116] Drugs.com. Enhertu. Available from: https://www.drugs.com/enhertu.html
[117] Drugs.com. Epkinly. Available from: https://www.drugs.com/epkinly.html
[118] Drugs.com. Fabhalta. Available from: https://www.drugs.com/fabhalta.html
[119] Drugs.com. Imfinzi. Available from: https://www.drugs.com/imfinzi.html
[120] Drugs.com. Livmarli. Available from: https://www.drugs.com/livmarli.html
[121] Drugs.com. Otezla. Available from: https://www.drugs.com/otezla.html
[122] Drugs.com. Rybrevant. Available from: https://www.drugs.com/rybrevant.html
[123] Drugs.com. Wegovy. Available from: https://www.drugs.com/wegovy.html
[124] Drugs.com. Xolair. Available from: https://www.drugs.com/xolair.html
[125] Drugs.com. Zepbound. – [Электронный ресурс]. – Режим доступа: https://www.drugs.com/zepbound.html

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the pharmaceutical industry shows progress in the development and registration of innovative medicines aimed to develop targeted and biological medicines. The dynamic development of the biologics industry and, in particular, mAbs aimed at immunotherapy of cancers, reflects the transition from chemotherapy to immunotherapy. At the same time, the use of mAbs is not limited to this applied point: mAbs can be used to treat hemophilia and Alzheimer's disease. The observed trend has important applied and fundamental significance. The applied significance lies in the need to develop technologies and train personnel to create medicines based on the interaction of exogenous (xenobiotic) and endogenous macromolecules — receptors, factors, enzymes, ion channels, and their ligands. The fundamental significance of the growth in the share of biologics among the first-in-class lies in the need for a comprehensive study of the pathological mechanisms of widespread and rare diseases, with an emphasis on the role of protein factors.

We also note the important role of repurposing registered medicines. Despite the fact that in most cases, additions of indications do not imply a fundamentally new use, the development of a new dosage form or the identification of effectiveness against a type of cancer previously not indicated may benefit practical healthcare. A change in the dosage form, expanding the age range of patients, and the inclusion of a new form of cancer in the indications contribute to an increase in the number of patients for whom a drug is available, the clinical development of which has been completed, and production has already been established. Moreover, in many cases, a medicine with a new indication turns out to be a breakthrough therapy, from which one should not underestimate the repurposing of known medicines as a developed tactic.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS CONTRIBUTION

Denis V. Kurkin — idea and planning of the structure of the work, design of graphic material, editing and approval of the final version of the manuscript; Nazar A. Osadchenko Anastasia R. Makarova, Dmitry A. Bakulin, Olga V. Marincheva, Yuliya V. Gorbunova, Dina V. Yunina, Ksenia N. Koryanova, Valentina I. Zvereva — collection of material and writing draft of the manuscript; Marina A. Dzhavakhyan, Olga O. Shatalova, Evgeny I. Morkovin, Andrey V. Strygin, Yury A. Kolosov — editing of the final version of the manuscript; Andrey V. Zaborovskiy, Vladimir I. Petrov, Roman V. Drai, Daria A. Galkina, Igor E. Makarenko, Anna S. Shuvaeva — consultations on highly specialized issues, approval of the final version of the manuscript. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication).

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