REPRODUCTION AND APOPTOSIS OF EBV-LATENT INFECTED CELLS UNDER INFLUENCE A TRIZ-CREATED ANTIVIRAL DRUGS

Farber[1] B, Martynov[2] A, Klein[1] I.

[1] Noigel LLC
[2] Mechnikov Institute of Microbiology and Immunology

Introduction. More than 30 years ago, we discussed the prospects of TRIZ application (the theory of inventive problem solving in medical drug development with Genrich Altshuller, who is creator of the TRIZ. TRIZ is a problem-solving, analysis and forecasting philosophy of thinking derived from the study of patterns of invention and theory evolution. While TRIZ has been used widely in different fields of human life, mainly fields of technology, there is one field where it has not been utilized, pharmaceutical and medical fields.

This is an area which affects almost every family, and each and every one of us. Namely, the development of new effective and harmless drugs. Our company first in the world focuses on the application of TRIZ in drug development to create synergistic combinations of existing drugs and discover their unique function, as well as, designing new drugs with non-standard properties. As a result, based on principles of TRIZ and computerized mathematical modeling, we have created a line of new dynamic, quasi-living, self-organizing medical drugs. According to TRIZ there are several main laws of system development: Increase the degree of ideality; Irregular evolution of system parts & coordination; Increase the degree of system dynamics; Increase of manageability of the system; Transition of a system to a super system. In order to improve their performance, rigid systems should become more dynamic: evolve to more flexible and rapidly changing structures, adaptable to changes of working conditions and requirements of the environment. We have applied its principles for many years to solve various problems. As a result, in late 80's we came up with the idea of "self-organizing" dynamic drugs. Drugs dynamic features would allow them the possibility of adapting to each specific medical disease- the first goal was to target anti-cancer and antiviral by liposomal drugs. One aspect of approach was the idea of a plurality of components (the combinatorial principle). We made analogy with a set of master keys for different locks (a universal combinatorial antiviral drug) would be preferred over the common approach, one key for one lock (one drug for one receptor).

We had worked persistently and in 1997, the first model of such a structure based on oligopeptides of horse albumin was revealed. We named this project and called Albuvir (combined from the word Albumin and the word virus) and applied in veterinary medicine, where viral infections represent more than 95% of all pathologies. In 2000 an application was filed for the first modification of this drug.

Subsequently, for the production and sale of this product, registration certificates of the State Committees in Veterinary Medicine of Eastern European countries were obtained.

In 2010, we signed a license agreement to transfer of limited rights in the field of veterinary medicine for the production and sale of Albuvir in Eastern Europe. The drug was produced commercially and was successfully used to cure hundreds of thousands of chickens, rabbits, sheeps, cows, pigs, pigeons, fish (industrial - sturgeon), cats, dogs, geese, ducks, and other types of farm birds and animals.

Over the years, we have made significant progress to improve Albuvir effectiveness. Our new, much stronger modifications, has shown that it works successfully on multiple different viral infections and has a minimum of side effects. Since the effectiveness of Albuvir increased, we even managed to reduce the therapeutic dose of the drug and increase the bioavailability and speed of the first manifestations of the pharmacological effect. In order to check effectiveness of the drug, we study influence of the drug on the reproduction and apoptosis of cells latently infected with the Epstein-Barr virus in vitro.

The Epstein-Barr virus (EBV) is a DNA-containing herpesvirus that has the ability to persist for life in the host [1]. It was previously thought to be a safe virus that can sometimes cause mononucleosis in humans [2].

Later evidence emerged that EBV has oncogenic properties. Clear correlations were established between EBV and a number of tumors, in particular - nasopharyngeal adenocarcinoma, lymphogranulomatosis, gastric cancer, esophageal cancer [3]. Recently, there are more links and articles related to triggering role of this virus in the development of atherosclerosis and in the development of chronic lifelong immunodeficiency [4]. The latter is associated with a high lymphotropicity of EBV, especially towards B-lymphocytes and plasma cells. With latent EBV infection, the production of high avidity immunoglobulins for almost all pathogens is significantly reduced. This causes both a hyperfunction of the immune system in the form of allergies such as asthma, skin allergies and autoimmune pathologies such as lupus and rheumatoid arthritis, with obvious background of a PCR test positive for EBV (more than 105 DNA copies / ml) [5]. Recently, more and more scientists consider EBV to be the cause of the development of multiple autoimmune disease, incl. allergic pathologies. EBV also contributes to severe inflammatory processes of the central and peripheral nervous systems, causing meningoencephalitis, meningitis, neuritis and arachnoencephalitis [6]. Therefore, the development of drugs that would contribute to the elimination of the EBV pathogen both in the phase of active reproduction and in the latent phase is an urgent task of modern virology. Recently, many novel therapeutics have been studied to eliminate latently affected cells through apoptosis, through the activation of the expression of latent EBV proteins in combination with standard drugs. [7]. This is a group of drugs - activators of histone decetylase [8]. One of the most interesting drugs in this novel group is the fairly widespread drug valproic acid and its derivatives. The valproates have been shown, activation and transfer of the latent form of EBV into the active phase. In the active phase, this virus is quite well treated with acyclovir derivatives (ganciclovir). The combination of valproic acid with ganciclovir and anticancer agents showed a statistically significant increase in the effectiveness of treatment of certain types of cancer in the

DOI: 10.5281/zenodo.4038923

clinic [9]. In our study, we studied the effect of the dynamic antiviral agent Albuvir on the latent and active phase of EBV viral reproduction. Albuvir is a modified oligopeptide based on the nuclear localization signals of importins. Its use not only suppresses the reproduction of the virus, but is also able to stimulate the apoptosis of latently infected cells [10]. It is also able to adapt to viruses and cells of every organism, having the ability to self-assembly and self-organization on signal peptides of nuclear localization and nucleolar polyribosomes. Drug development is one of the most important research areas, which affects each one of us. However, nobody has used TRIZ as a philosophy of solving problems in pharmaceutical research and development. The application of the principles of TRIZ in this arena opens up broad prospects in the creation of new classes of drugs that can independently adapt to the patient's body.

The combination of contradictions, laws of development systems, algorithms, Su-field analysis, TRIZ principles, deep fundamentals of pharmaceutical industry and pharmacology, modern computer mathematical modeling, in the solution of each of the tasks at once, allows us to achieve extraordinary results and obtain significantly more effective novel drugs. For the first time in the world we have developed dynamic self-organizing, quasi live drugs, based on the principles of TRIZ and computerized mathematical modeling [11,12,13].

These drugs capable of adapting independently both to the human body and to molecular targets, including viruses, cancer cells and microorganisms. Applying TRIZ and mathematical modeling in pharmaceutical industry, produces novel and future R&D trends. This increased the yield of novel dynamic drugs [14]. The dynamic drugs can overcome many problems from resistance to the slippage effect, to eliminate the side effects of drugs [15].This will save millions of lives. Despite changes in the concept of drug development: from banal screening (out of thousands of synthesized compounds, only one showed biological activity) to those obtained as a result of molecular modeling (another name is drug-design).

Albuvir is composed of a mixture of acylated peptides. It effectively inhibits the process of nuclear importation of viral polynucleotides from those viruses that depend on the cell nucleus (FLU, Herpes Viruses, HIV/AIDS) [16]. Currently Albuvir is widely used as a veterinary drug on the market for the treatment of animals infected with viruses and for the prevention of animal mortality from excessive replication of vaccine viruses during vaccination [17,18]. The mechanism of action of Albuvir is based on the temporary inhibition of nuclear import peptides (alpha- and beta-importins) [19]. Carboxylated Albuvir peptides bind to the nuclear import peptide signal, and block both viral genome penetration into the cell nucleus and the release of viral particles of DNA from the nucleus. At the same time, on the target (imported), the active inhibitor self-assembles from various inactive Albuvir peptides. Albuvir pharmacological indicators: LD_{50} = 2880 mg / kg, ED_{50} = 25 mg / kg, Ti = 115.2, $T_{1/2}$ = 29 min, application method: per oral. Albuvir has antiviral activity for Influenza virus, Herpes viruses Types 1 and 2, Human Cytomegalovirus, Herpes Zoster virus, Epstein-Barr virus, Coronavirus. What is the TRIZ contradiction? On one side – drugs are static substances and cannot effectively use one drug treatment of multiple viral infections, on the other, different viruses have many different signals of importins and for treatment of those viral infections we need many different drugs (one virus– one drug). It means our one drug must be "smart", substitute many drugs and self-adjust to multiple viruses. This contradiction was resolved by our company using the principles of TRIZ.

The idea of creating Albuvir was based on principles of TRIZ: fragmentation (to divide an object into independent fragments — fragmentation of whole proteins into oligopeptides with replacement of their charge); the principle of dynamism:

a) the characteristics of the object should be changed so as to be optimal at each stage - for different viruses and different sequences of nuclear import peptide signals, a different sequence of inhibitor from a mixture of Albuvir peptides;

b) to divide the object into parts capable of moving relatively to each other - fragmentation of proteins to Albuvir oligopeptides); the principle of copying (instead of an inaccessible, complicated, expensive, inconvenient or fragile object to use its simplified and cheap copies - instead of synthesizing a highly specific and expensive non-peptide inhibitor of nuclear import signals, use a mixture of oligopeptides with different sequences from available raw materials);

c) the principle of homogeneity (objects interacting with this object should be made from the same material or close to it in properties - instead of synthesizing complex highly specific xenobiotic, use fully biodegrading peptides from natural sources, but with preliminary replacement from charges with opposite ones); the principle of rejection and regeneration of parts (having fulfilled its purpose or become an unnecessary part of an object must be discarded (dissolved, evaporated, etc.) or modified directly during the work - complete biodegradation of "spent" and excessive concentrations of Albuvir peptides without toxic metabolites;

d) the principle of dissociation-association ("Dissociation-association" is stronger than the "separation-association". It allows the substance to split apart when necessary, and when it is necessary to turn into one substance again — the Albuvir complementary peptides are associated with nuclear import peptides);

e) the principle of self-organization (self-assembling of an active substance from inactive precursors - out of thousands of synthesized Albuvir peptides, only a small number of high-affinity fragments will be associated with the signal peptides of beta-importins, and for each virus these will be their peptides) [20,21]

Thus, the task of this work was to study the ability of Albuvir to inhibit EBV reproduction and induce apoptosis of latently infected cells in the culture.

Materials and methods.

Cell culture. The inhibitory effect of Albuvir on EBV was studied in the following cell cultures: Raji - cell culture of the B-phenotype of Burkitt's lymphoma (human lymphocytes transformed by EBV, which have 63 copies of the viral genome in their genome, and produce only

DOI: 10.5281/zenodo.4038923

individual early antigens, and not whole viral particles). Namalwa is a B-phenotype cell culture from Burkitt's lymphoma that lacks the EBV genome. B95-8 is a marmoset monkey B-lymphocyte cell line containing the complete EBV genome, producing complete viral particles. Cell cultures were grown on a growth medium consisting of 90% RPMI 1640 medium (Sigma, USA), 10% solution of fetal calf serum (Sigma, USA), penicillin (100 μg / ml), streptomycin (100 μg / ml), L-glutamine (2 mM), in a thermostat at 37 ° C with the addition of 5% CO_2. To control cell viability, a 0.4% trypan blue dye (Sigma, United States) was used. EBV was isolated from a lymphoblastoid culture of B95-8 cells (B-lymphocytes of monkeys-marmazetka), which produces this virus, according to the method of Walls, Crawford [22].

Investigated substances. Albuvir (N. 1) - dynamic acylated acidic peptides - antagonists of signal peptides of nuclear (nucleolar) localization and polyribosomes. Lysine trisuccinamide (N. 2). Acyclovir was used as a reference drug (literature data) (Acycloguanosine (lot 117F0756), m.v. 225.2 (Sigma, USA)) (N. 2) [23]. HPLC characteristics of Albuvir are shown in Fig. 1. The test substances were dissolved in RPMI-1640 medium, filtered through sterilizing filters from Sarstredt, Germany, with a pore size of 0.22 μm. Working dilutions were prepared in growth medium (95% RPMI-1640 medium with 5% fetal calf serum).

MTT test. The cytotoxic concentration was determined in the Raji lymphoblastoid cell system using the colorimetric MTT method. This method is widely used to determine the CC50 of potential drugs in the study of the cytopathic action of viruses in vitro. MTT (3, (4,5 dimethyltriazol-2-yl) -2,5-diphenyltetrosolium bromide) was dissolved in sterile phosphate-buffered saline (PBS) (pH 7.4) at room temperature to a concentration of 5 mg / ml and filtered (0.22 micron filters "Sarstredt", Germany). Special attention was paid to the standardization of experimental conditions. Raji cells were plated into 96 well culture plates, 100 μl per well, 25 μl MTT (final concentration 5 μg / ml) was added and incubated for 3 h at 37 ° C in an atmosphere of 5% CO2. After incubation, cells were washed with PBS and resuspended in 96% ethanol to dissolve formazan. The results were analyzed spectrophotometrically on a Dynatech reader (Sweden) at a wavelength of 540 nm.

Polymerase chain reaction. The degree of influence of drugs on EBV reproduction was determined using PCR test systems "AmpliSens-100-R". A fragment encoding the VCA protein of the virus with a size of 290 nucleotide sequences was the chosen genome region of the Epstein-Barr virus. The control was cells that, after infection with the virus, were incubated in the growth medium without the addition of the substances that were studied. We determined the percentage of inhibition of the level of accumulation of viral DNA in the samples treated with the test substances in relation to the control sample, the value of which was taken as 100%.

Statistical Methods. According to the obtained research results, statistical analysis was carried out using standard methods [24]. To determine the CC_{50} and IC_{50} indicators, we used the regression analysis method (using the Microsoft Excel computer program), which allows us to characterize the relationship between two signs, namely, taking as a basis one of the signs (in our case, concentration) to assess the variation and the other indicator associated with it (optical density of the prototype).

Results and discussion

According to the results of the MTT method, the IC_{50} (the dose of the compound that leads to inhibition of the viability of 50% of cells in culture) was calculated, and the effect on the mitochondrial and lysosomal activities of cells was also investigated. Directly, the decrease in mitochondrial and lysosomal cell activity was determined by comparing the values of samples exposed to the action of the compounds for 48 hours with the values of the control cells.

The decrease in mitochondrial activity under the action of compound N. 1 of B95-8 cells is shown in Fig. 1.

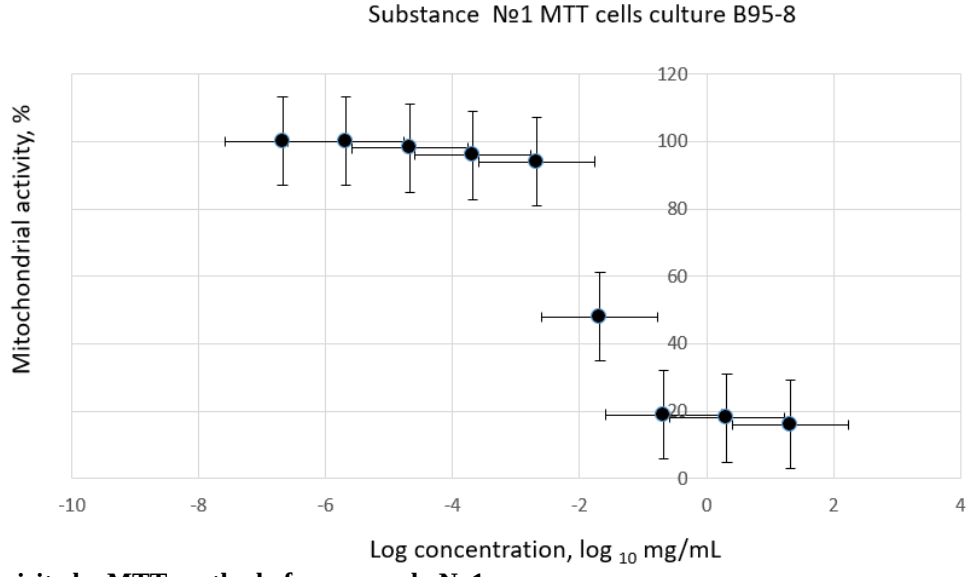

Fig. 1. Cytotoxicity by MTT-method of compound N. 1.

DOI: 10.5281/zenodo.4038923

Concentrations correspond to table 1.

As can be seen from Figure 1, a 50% drop in the mitochondrial activity of cells in culture is noticeable already in the presence of substance N. 1 at a concentration of 0.07 ug/mL. At high concentrations of Albuvir (N. 1), the activity of mitochondria in cells drops to 20%.
The decrease in mitochondrial activity under the action of compound N. 2 of B95-8 cells is shown in Figure 2.

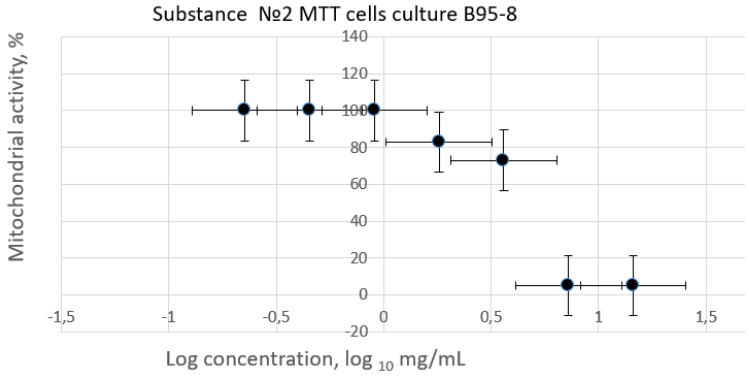

Fig. 2. Cytotoxicity by the MTT method of compound N. 2 based on a decrease in mitochondrial activity.

Compound N. 1 turned out to be the most cytotoxic, while compound N. 2 turned out to be less toxic. The IC_{50} value is shown in Table 1.

Table 1. The IC_{50} for researched substances

Index	IC_{50}, mg/mL	
Culture	B95-8	
Compound	№1	№2
MTT method	0,0068	2,5

Inhibition of the lysosomal system was also dose-dependent. The toxicity was tested at the doses indicated in Tables 1 and 2 for the B95-8 culture. Compound N. 1 was almost 3 orders of magnitude more active in the MTT test than compound N. 2 according to IC_{50}
The nature of suppression of the lysosomal system of B95-8 cells is shown in Figure 3 for compound N. 1.

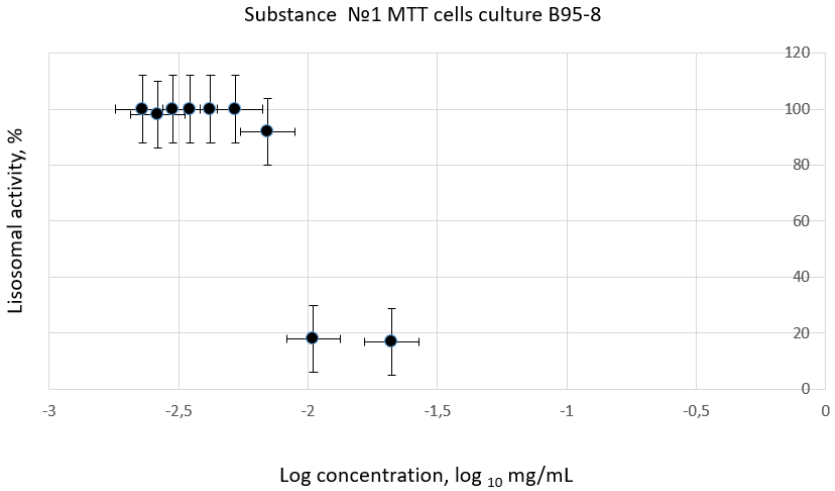

Fig.3. Cytotoxicity by the neutral red method for compound N. 1 and culture B95-8; concentration of compound N. 1 from table 2.

As can be seen from Figure 4, a drop to 20% of the lysosomal activity of the B95-8 cell culture for compound N. 1 was observed at a concentration of -2 log10 m mg / mL, respectively, the cytotoxicity against infected EBV cells in the latent phase is quite noticeable in comparison with the control - acyclovir. The latter did not have any significant cytotoxic effect on latently infected cells at all.
The inhibition of the lysosomal system by compound 2 is shown in Figure 4.

DOI: 10.5281/zenodo.4038923

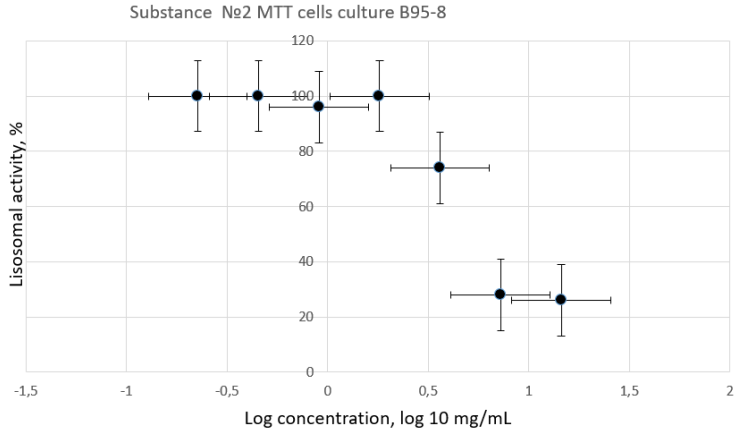

Fig.4. Cytotoxicity by the neutral red method for compound N. 2 and culture B95-8. IC_{50} indices by the method of neutral red culture B95-8 are shown in Table 2.

Table 2. The IC_{50} for researched substances

	IC_{50}, mg/mL	
	B95-8	
Substance	№1	№2
NR method	0,0079	1,94

To confirm the data obtained by the MTT and NR method for compound N. 1, the number of dead cells was counted using the trypan blue method. The result is shown in Figure 5.

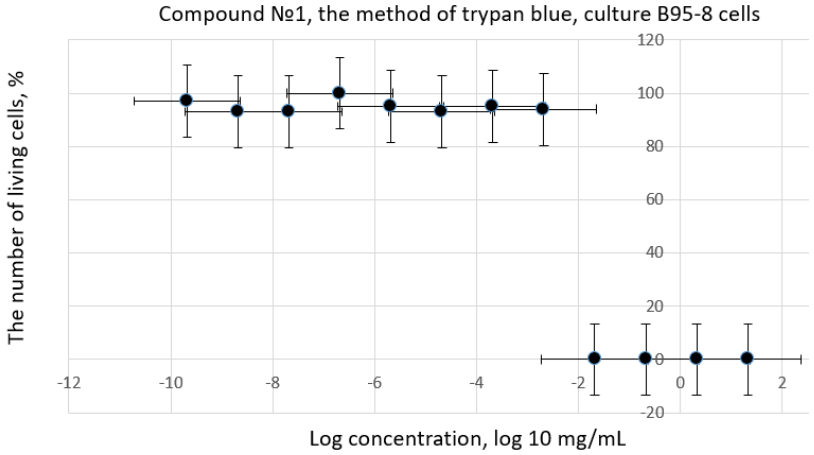

Fig. 5. Trypan blue cytotoxicity for compound N 1.

It should be noted that compound N. 1 at a concentration of 2.1% (21 mg / ml) led to complete cell lysis, and can also interact with the artificial MTT substrate, staining it purple.

The study of compounds for cytotoxicity for the BHK-21 culture revealed a significantly lower toxicity of the compounds for this culture.

Studies were conducted for the culture of VNK-21 in the concentrations given in Table 3.

Table 3. Substance study onVNK-21 in the different concentrations

Substance N 1			Substance N 2		
Dilution	Concentration, mg/ml	Log 10, mg/mL	Dilution	Concentration, mg/mL	Log_{10}, mg/mL
1^640	0,328	-0,4841262	1^640	0,453	-0,3439018
1^320	0,656	-0,1830962	1^320	0,906	-0,0428718
1^160	1,3125	0,11809931	1^160	1,8125	0,25827802
1^80	2,625	0,41912931	1^80	3,625	0,55930801
1^40	5,25	0,7201593	1^40	7,25	0,86033801
1^20	10,5	1,0211893	1^20	14,5	1,161368
1^10	21	1,32221929	1^10	29	1,462398

DOI: 10.5281/zenodo.4038923

Inhibition of mitochondria activity by the composition N.1 for the culture of VNK-21 is shown in Figure 6.

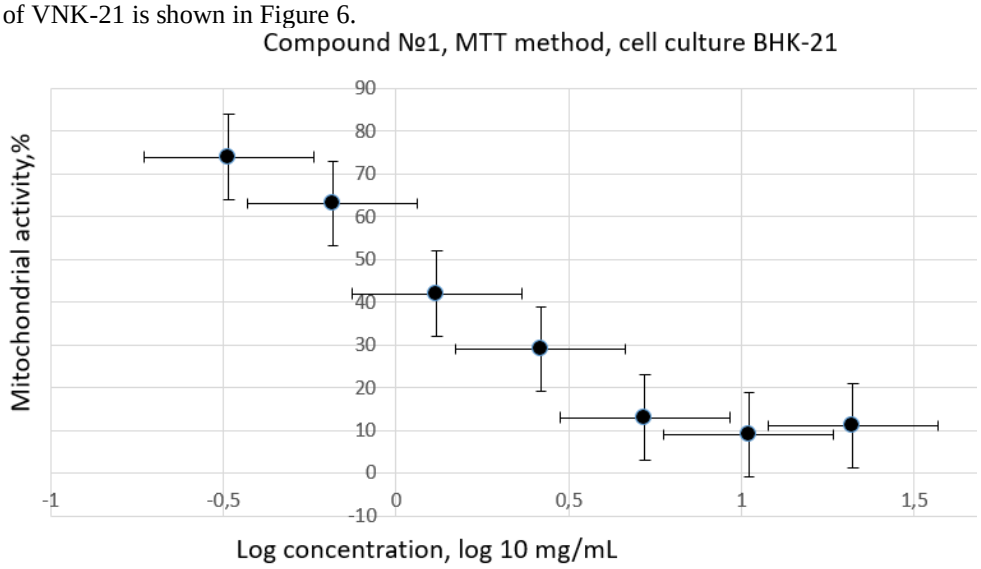

Fig. 6. Cytotoxicity by MTT method for compounding N.1 and culture of VNK-21.

The decrease in mitochondria activity by the composition number 2 for the culture of VNK-21 is shown in Figure 7.

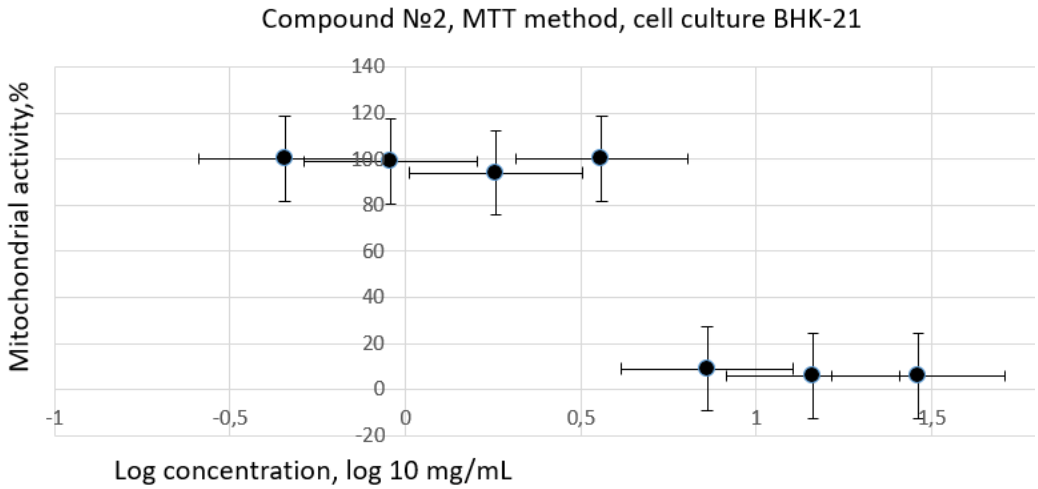

Fig. 7. Cytotoxicity by MTT method for compounding N.2 and culture of VNK-21.

A dose-dependent type of action of compounds on the mitochondrial activity of VNC-21 culture cells has been established, and IC50 has been calculated for this culture (see Table 4).

Table 4. IC_{50} studied compounds for VNK-21 cell culture

	IC_{50}, mg/mL	
	VNK-21	
Substance	№1	№2
MTT	1,06	3,5

As for the culture of B95-8, the compound N.1 was more toxic than control.The ability to stimulate apoptosis by the composition N.1 has been tested by the staining of cells with the fluorescent dye Hoechst 33342, which intercalates in the DNA of both living and dead cells, and the morphological state of the nucleus can determine the apoptotic or necrotic state of cells, as well as its normal state.

Compound number 1 was tested for the ability to stimulate apoptosis in 3 concentrations with exposure at 4:00 on the culture of B95-8. The results are in Table 5.

DOI: 10.5281/zenodo.4038923

Table 5. Effect of different concentrations of substance N. 1 (Albuvir) on the death of B95-8 culture cells

Concentration, mg/mL	Number of cells, %			Counted cells total number
	Normal	Apoptotic	Necrotic	
0,0046	64	22	14	100
0,0021	73	19	8	142
0,00021	81	11	8	100
Cell control	95	-	5	81

The ability to induce apoptosis is also dose-dependent, with a maximum rate of 22%. Fig. 8-9 shows photos taken with fluorescent microscopy.

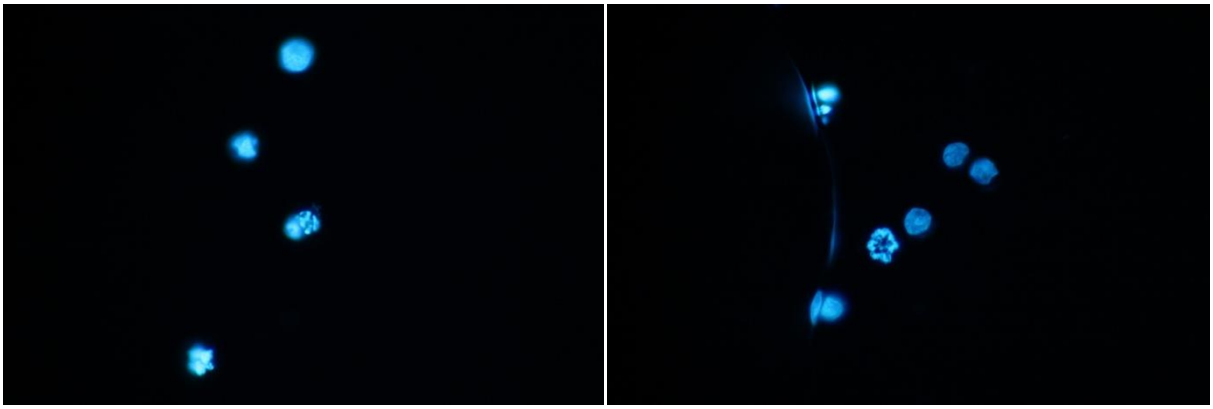

Fig.8. Photographs are obtained in fluorescent microscopy, which depict normal and apoptotic cells (there is a defracation of the nucleus). Increase of 100, ultraviolet with blue filter.

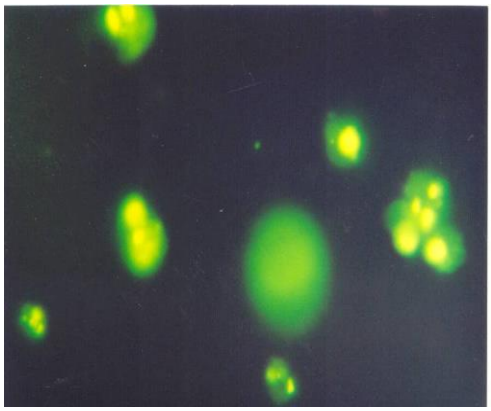

Fig.9. Photographs were obtained with fluorescent microscopy, which depict normal and apoptotic cells (there is a deframentation of the nucleus). Increase of 900

Thus, the indicators of cytotoxicity N1, N2 and reference of the drug Acyclovir MTT-method, which includes colorimetric detection of living cells, have been determined. Using linear regression analysis, the indicators of IC_{50} were calculated, which amounted to: for N1 - 1.06 mg/mL, substances N2 - 3.5 mg/mL, for Acyclovir - 5 mg/mL.

To determine the antiviral action of each sample under study, an inhibitory concentration (IC_{50}) was determined, i.e. concentration, reducing reproduction of the virus by 50%. The drug N. 1 was studied in concentrations of 0.1, 0.5, 1, 5, 10 ug/mL. Each concentration was investigated in three repetitions. The drug remained in the environment throughout the study cycle.

The polymerase chain reaction method was used to determine the level of EBV reproduction in the cells studied. The analysis of the antiviral action of the drugs was carried out after 48 hours, as this time point is optimal both in terms of the dynamics of the growth of the Raji cell line and the reproductive cycle of EBV.

Antiviral activity has been established that the IC_{50} Albuvir (N1) is 5 ug/mL, for reference-drug Acyclovir - 222 ug/mL.

DOI: 10.5281/zenodo.4038923

Table 6. The level of inhibition of reproduction of the Epstein-Barr virus under the influence of various concentrations of the experimental drugs by PCR method.

Concentration of ug/ml	The level of inhibition of reproduction of the Epstein-Barr virus (%)	
	Albuvir	Acyclovir
0,1	20	0
0,5	50	0
1	56	0
5	67	0
10	80	10

So, it is determined that Albuvir in the range of experimental concentrations has low toxicity in the culture of raji cells. A 50% decrease in proliferative activity is observed at 840 ug/mL of the substance, which corresponds to the value of CC_{50}. For Acyclovir CC_{50} was 5000 micrograms / ml. When observing the antiviral activity of the studied drugs in the culture of Raji cells, the effective concentration EC_{50}, that is, the concentration that reduces the reproduction rate of the Epstein-Barr virus by 50% is 1 ug/mL, for reference drug Acyclovir - 222 ug/mL. The Selectiveness Index (SI) was determined by the ratio of CC_{50} to the EC_{50}. SI Albuvir was 8400 and reference-drug Acyclovir was 22.6. Antiviral drugs that have SI above 16 when researching the inhibitory effects on reproduction of the virus in vitro systems are considered active and may be recommended for further animal research. It has been shown that Albuvir has the maximum antiviral activity compared to the reference drug Acyclovir and it may be a promising drug for the treatment of diseases caused by EBV. Despite the different development of viral infection in different diseases depending on the localization in the body, we considered it appropriate to conduct studies aimed at the interaction of this drug and the virus in different cell cultures. The Namalwa cell line, which does not contain the Epstein-Barr virus genome, was used for research, as a line where the abortive replication of the virus occurs according to a latent scheme and a B95-8 cell culture - a cell line of B-lymphocytes of marmoset monkeys containing the complete EBV genome and produces complete viral particles.

According to the above methodical approaches, the cytotoxic and antiviral action of Albuvir in B95-8 cells was analyzed. It is determined that in the range of concentrations of 50-500 ug/mL this drug has little effect on the viability of cells. The concentration, which reduces the viability of the cellular population by 50%, is about 1250 ug/mL. In the minimum studied concentration of 0.5 mcg/ml, up to 20% of the inhibition of the reproduction of the virus is observed. Thus, the B95-8 cell culture is insensitive to the cytotoxic action of the Albuvir, and it is an active inhibitor of EBV reproduction. The index of selectivity in this system for Albuvir was 500 as a result of the determination of its anti-EBV activity in the culture of cells Namalwa by PCR method found that the concentration of 50 ug/mL reduces the level of accumulation of EBV DNA by 50%. Based on its cytotoxic concentration in this cell culture, which is 3000 ug/mL, the selective index is 60. In this culture of cells anti-EBV activity is moderate.

In the table. 7 is induced by the CC_{50}, EC_{50} ta SI for Albuvir in the cell cultures B95-8 and Namalwa.

Table 7. Comparison of SS50 and EU50 for PV-1 in B95-8 and Namalwa cell cultures.

The studied indicators	**Cell culture**	
	B95-8	**Namalwa**
CC_{50}	1250 ug/ mL	3000 ug/mL
EC_{50}	2,5 ug/ mL	50 ug/ mL
IS	500	60

Few thoughts about future drug development. The cost of developing new drugs grow, and the return on investment for drug development is decreasing every year. At the same time, expenses on research and development (R&D) are gradually increasing each year. The development of drugs is more time-consuming, while being more expensive, despite recent technological improvements. New technological advancements led to more precision in quantitative and qualitative control of drug research. This trend has been observed from the 50s to the present, and the cost of developing a new drug has doubled approximately every ten years.

According to Genrich Altshuller [25], the life of a technological system (as incidentally in other systems, including biological) can be presented in the shape of an S-curve, showing how the main characteristics of a system change in time. In different systems this curve naturally has its own individual features but it always has characteristic segments, which are brought out schematically with crudely depicted emphasis. In "childhood " the system develops slowly. Then the time of

DOI: 10.5281/zenodo.4038923

"growing up" and "maturity» comes - the system is quickly improved, and its mass application begins. At some point the rates of development start to fall and "old age" sets in. Later two variants. One system either degrades, changing into another, a system that is different in its principle, or for a long time stick to achieved values. Based on TRIZ, we could think about some future evolution of medicine and pharmaceuticals to Drugs dynamization as a future pharmaceutical industry – this are drugs with elements of a living organism, meaning it is capable of adapting to the disease mutations and the body of a particular person, without or with very minimal side effects.

Conclusion

Effect of the dynamic antiviral drug Albuvir on reproduction and apoptosis latently infected with the Epstein-Barr virus cells in vitro. Modern approaches to the treatment of herpes infection, in particular the Epstein-Barr virus (EBV), include the use of ethyotropic drugs, as well as sensitizing therapy. This virus plays an important role in the etiology of nasopharyngeal carcinoma, stomach carcinoma, Burkitt lymphoma and lymphoproliferative syndromes. The range of drugs active against EBV remains very limited to ganciclovir and acyclovir. The search for new compounds active against EBV remains relevant. The aim of this study was to find out the anti-EBV activity of the drug Albuvir in Raji, B95-8, Namalwa lymphoblastoid cells. The cytotoxicity concentration (CC_{50}) was determined, which was 3000 ug/mL, and the concentration of the drug that inhibits the reproduction of the virus (EC_{50}) was 0.1 ug/mL. The ability of the drug Albuvir to inhibit the reproduction of the Epstein-Barr virus in all studied cell cultures was revealed. When the economic efficiency of creating static drugs in accordance with the S-shaped curve decreases, the need arises for a transition to a supersystem, namely, the creation of dynamic drugs systems.

It has been proven that the drug Albuvir is able to inhibit the reproduction of the Epstein-Barr virus in Raji, B95-8 and Namalwa cell cultures. It is determined that the drug has a high activity in the culture of Raji cells (SI 8400), respectively, the drug is promising enough to develop as a treatment for EBV-associated diseases.

Acknowledgments

We are grateful to the Ph.D., Svetlana Zagorodnaya, head of the virus reproduction department of the Microbiology and Virology of the D.K. Zabolotny Institute of Microbiology and Virology National Academy of Sciences of Ukraine for providing assistance in conducting virological studies of the developed drug.

Conflict of Interests

No conflict of interests

Reproduction and apoptosis of EBV- latent infected cells under influence a TRIZ-created antiviral drugs

Farber B, Martynov A, Klein I.

Introduction. We had worked persistently and in 1997, the first model of such a structure based on oligopeptides of horse albumin was revealed. We named this project and called Albuvir (combined from the word Albumin and the word virus) and applied in veterinary medicine, where viral infections represent more than 95% of all pathologies. In 2000 an application was filed for the first modification of this drug. Subsequently, for the production and sale of this product, registration certificates of the State Committees in Veterinary Medicine of Eastern European countries were obtained. In 2010, we signed a license agreement to transfer of limited rights in the field of veterinary medicine for the production and sale of Albuvir in Eastern Europe. The drug was produced commercially and was successfully used to cure hundreds of thousands of chickens, rabbits, sheeps, cows, pigs, pigeons, fish (industrial - sturgeon), cats, dogs, geese, ducks, and other types of farm birds and animals. Thus, the task of this work was to study the ability of Albuvir to inhibit EBV reproduction and induce apoptosis of latently infected cells in the culture.

Materials and methods. Cell culture. The inhibitory effect of Albuvir on EBV was studied in the following cell cultures: Raji - cell culture of the B-phenotype of Burkitt's lymphoma. Namalwa is a B-phenotype cell culture from Burkitt's lymphoma that lacks the EBV genome. B95-8 is a marmoset monkey B-lymphocyte cell line containing the complete EBV genome, producing complete viral particles. To control cell viability, a 0.4% trypan blue dye (Sigma, United States) was used. EBV was isolated from a lymphoblastoid culture of B95-8 cells (B-lymphocytes of monkeys-marmazetka), which produces this virus, according to the method of Walls, Crawford. Investigated substances. Albuvir (N. 1) - dynamic acylated acidic peptides - antagonists of signal peptides of nuclear (nucleolar) localization and polyribosomes. Lysine tris-succinamide (N. 2). Acyclovir was used as a reference drug (references data) MTT test. The cytotoxic concentration was determined in the Raji lymphoblastoid cell system using the colorimetric MTT method. This method is widely used to determine the CC50 of potential drugs in the study of the cytopathic action of viruses in vitro. Raji cells were plated into 96 well culture plates, 100 μl per well, 25 μl MTT (final concentration 5 μg / ml) was added and incubated for 3 h at 37 ° C in an atmosphere of 5% CO_2. After incubation, cells were washed with PBS and resuspended in 96% ethanol to dissolve formazan. The results were analyzed spectrophotometrically on a Dynatech reader (Sweden) at a wavelength of 540 nm. Polymerase chain reaction. The degree of influence of drugs on EBV reproduction was determined using PCR test systems "AmpliSens-100-R". A fragment encoding the VCA protein of the virus with a size of 290 nucleotide sequences was the chosen genome region of the Epstein-Barr virus. The control was cells that, after infection with the virus, were incubated in the growth medium without the addition of the substances that were studied. We determined the percentage of inhibition of the level of accumulation of viral DNA in the samples treated with the test substances in relation to the control sample, the value of which was taken as 100%. **Results and discussion.** Effect of the dynamic antiviral drug Albuvir on reproduction and apoptosis latently infected with the Epstein-Barr virus cells in vitro. Modern approaches to the treatment of herpes infection, in particular the Epstein-Barr virus (EBV), include the use of ethyotropic drugs, as well as sensitizing therapy.

DOI: 10.5281/zenodo.4038923

The range of drugs active against EBV remains very limited to ganciclovir and acyclovir. The search for new compounds active against EBV remains relevant. The aim of this study was to find out the anti-EBV activity of the drug Albuvir in Raji, B95-8, Namalwa lymphoblastoid cells. The cytotoxicity index (CC50) was determined, which was 3000 ug/mL, and the concentration of the drug that inhibits the reproduction of the virus (EC_{50}) was 0.1 ug/mL. The ability of the drug Albuvir to inhibit the reproduction of the Epstein-Barr virus in all studied cell cultures was revealed. When the economic efficiency of creating static drugs in accordance with the S-shaped curve decreases, the need arises for a transition to a supersystem, namely, the creation of dynamic drugs systems. It has been proven that the drug Albuvir is able to inhibit the reproduction of the Epstein-Barr virus in Raji, B95-8 and Namalwa cell cultures. It is determined that the drug has a high activity in the culture of Raji cells (SI 8400), respectively, the drug is promising enough to develop as a treatment for EBV-associated diseases.

Keywords: TRIZ, laws of system evolution, dynamization, S-curve, Epstein-Barr virus, cell culture, antiviral action, albumin.

References

1. Hutt-Fletcher L.M. The Long and Complicated Relationship between Epstein-Barr Virus and Epithelial Cells. J Virol. 2017. 91.1. e01677-16-. doi: 10.1128 / JVI.01677-16
2. Dunmire S.K., Verghese P.S., Balfour H.H. Primary Epstein-Barr virus infection. J Clin Virol. 2018. 102. P. 84-92. doi: 10.1016 / j.jcv.2018.03.001
3. Kenney S.C., Mertz J.E. Regulation of the latent-lytic switch in Epstein-Barr virus. Semin Cancer Biol. 2014. 26. P. 60-68. doi: 10.1016 / j.semcancer.2014.01.002
4. Ibrahim A.I., Obeid M.T., Jouma M.J., et al. Detection of herpes simplex virus, cytomegalovirus and Epstein-Barr virus DNA in atherosclerotic plaques and in unaffected bypass grafts. J Clin Virol. 2005. 32 .1. P. 29-32. doi: 10.1016 / j.jcv.2004.06.010
5. Balandraud N., Roudier J. Epstein-Barr virus and rheumatoid arthritis. Jt Bone Spine. 2018. 85.2. P. 165-170.
6. Kononenko V.V. Epstein-Barr virus infection with lesions of the nervous system: clinical features, diagnosis, classification and treatment // Neurosurgical Journal. 2003. 1. P. 105-110.
7. Kenney S.C., Mertz J.E. Regulation of the latent-lytic switch in Epstein-Barr virus. Semin Cancer Biol. 2014. 26. P. 60-68. doi: 10.1016 / j.semcancer.2014.01.002
8. Ghosh S.K., Perrine S.P., Williams R.M., Faller D.V. Histone deacetylase inhibitors are potent inducers of gene expression in latent EBV and sensitize lymphoma cells to nucleoside antiviral agents. Blood. 2012.119.4. P. 1008-1017
9. Jones K., Nourse J., Corbett G., Gandhi M.K. Sodium valproate in combination with ganciclovir induces lysis of EBV-infected lymphoma cells without impairing EBV-specific T-cell immunity. Int J Lab Hematol. 2010. 32.1.169-174. doi: 10.1111 / j.1751-553X.2008.01130.x
10. Farber B.S., Martynov A.V., Kleyn I.R. Creation of New Medical Drugs Based on Triz and Computer Mathematical Modeling. AMI. 2018.4. P. 15-34. doi: 10.5281 / zenodo.2547580
11. Martynov A., Farber B., Farber, S. Quasi-life self-organizing systems: based on ensembles of succinylated derivatives of interferon-gamma. Current medicinal chemistry. 2011. 18. 22. P. 3431-3436.
12. Martynov A. V., Farber B. S., Farber S. B., Kabluchko, T. V. Synthesis of the ensembles from succinylated interleukin-2 derivatives and their biological activity in vitro. ScienceRise, 2015. 11.14 (16). P. 25-30.
13. Martynov A., Didenko G., Farber B., Farber S., Cruts O. The anticancer activity of antisense micro RNA (fRNA) in combination with the lectin from Bacillus subtilis B-7025. Journal of Pharmacy and Pharmacology. 2018. 70(6). P. 732-739.
14. Martynov A., Bomko T., Farber B. S., Nosalskaya T. N., Kleyn I. Synthesis of dynamic riboflavin derivatives and the study of their ability to urease photoinactivation. Annals of Mechnikov Institute. 2019. 3. P. 44-49.
15. Martynov A. V., Bomko T. V., Nosalskaya T. N., Farber B. S., Farber, S. B. Oral long-acting pharmaceutical form of insulin on the basis of self-organizing kvasi-living system of combinatorial peptides. Annals of Mechnikov Institute. 2012. 2. P. 64-70.
16. Martynov A. V., Babkin N., Zheynova, N. Antiviral activity of the albuvir in models vesicular stomatitis virus and human herpes virus type 1 in vitro. Scientific Bulletin of Lviv National University of Veterinary Medicine and Biotechnology named Gzhytsky. 2011.2. N. 1. P. 181-184.
17. Zheynova N. M. Principles of specific prophylaxis of Marek's disease and bacterial diseases of various etiologies // Veterinary Medicine of Ukraine. 2014. №. 5. P. 13-14.
18 Albuvir, the First and Only New-Generation Anti-Viral Drug. https://slideplayer.com/slide/15110346/#
19. Martynov A., Farber B. S., Farber S. S. U.S. Patent Application N. 14/023,231. 2015.
20. Altshuller G., Al′tov G., Altov H. And suddenly the inventor appeared: TRIZ, the theory of inventive problem solving. 1996.Technical Innovation Center, Inc.
21. Altshuller G. 40 principles: TRIZ keys to innovation (Vol. 1). 2002. Technical Innovation Center, Inc.
22. Uollz E., Crawford D. Culturing B95-8 cells // lymphocytes. Methods. Moscow: Mir, 1990. P. 230-249.
23. Datta A.K., Colby B.M., Shaw J.E., Pagano J.S. Acyclovir inhibition of Epstein-Barr virus replication. Proceedings of the National Academy of Sciences. 1980. 77 (9). P. 5163-5166.
24. Lopach S.N., Chubenko A.V., Babich P.N. Statistical methods in biomedical research using Excel. 2001. 320 s.
25. Altshuller G. S. Creativity as an Exact Science. The Theory of the Solution of Inventive Problems. 1984 Gordon and Breach Science Publishers Inc. 382 pages.

Received: 01.06.2020
Published: 21.09.2020