IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 4 Ser. II (Jul. –Aug. 2022), PP 09-15
www.Iosrjournals.Org

Promising Use of the Combination of Xenogeneic Antitumor Vaccine and Preparation Izatizon in Malignant Tumors

Cruts O.O.[1] , Cruts V.O.[3], Zaika L.A.[2], Bolsunova O.I.[2], Potopalskyi A.I.[4] , Didenko G.V.[1]

[1-]*RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine*
[2-] *Institute of molecular Biology and Genetics of the NAS of Ukraine*
[3-]*Bukovinian State Medical University of the MOH of Ukraine*
[4]*-Iinstitute of Heals Promotion and Rebirth of People of Ukraine*

Abstract:
Background*: Tumor disease is a system disorder, approaches to its therapy should be combined and include not only the application of cytotoxic therapy (chemotherapy), but new biotherapeutic methods as well. This study was aimed at exploring antimetastatic effect of combined regimens with xenogeneic antitumor vaccine and Izatizon in the animals with the tumor process.*
Materials and Methods*: Antitumor effect of combined administration of xenogeneic antitumor vaccine and Izatizon at the tumor Lewis lung carcinoma growth model (LLC) in mice were assayed. LLC was inoculated intramuscularly into lower limb's thigh (by $0.5x10^6$ cells/animal). During the experiment, the dynamics of tumor growth was evaluated by the number and volume of metastases in the lungs. Gelatinase activity was determined by the SDS-electrophoresis method*
Results*: Antitumor effects of the combination of xenogeneic antitumor vaccine (chicken embryonic proteins that underwent biotransformation under the action of protein containing metabolite of B. subtilis B-7025) and preparation Izatizon was more beneficial comparing with using of these drugs in a mono regimen. These actions manifested as the inhibition of primary tumor node development and as a significant delay of the metastasis process. In tumor tissue homogenates of the treated animals the reduced numbed of gelatinases that have an important role in tumor process manifestation was reduced.*
Conclusion*: The experimental data demonstrate the need for antiviral preparation application on the background of tumor growth at combined application with other biotherapeutic agents.*
Key Words*: Xenogeneic antitumor vaccine, embryonic proteins, izatizon, antitumor immunity.*

Date of Submission: 21-07-2022 Date of Acceptance: 05-08-2022

I. Introduction

The increase in mortality rates of oncological patients has prompted researchers to look for new methods and agents of therapy [1]. As far as tumor disease is a system disorder, approaches to its therapy should be combined and include not only the application of cytotoxic therapy (chemotherapy), but new biotherapeutic methods as well. At present, it is known that some viruses can be etiological factors of tumor disease development [2, 3]. Therefore, for the therapy of oncological patients, the use of antiviral drugs that not only eliminate the virus infection but also significantly reduce to load on the immune system, is justified. Due to this approach the use of other biotherapy methods should be more effective. Rather effective biotherapy approach is the use of antitumor vaccines. To-date, antitumor vaccines, developed at the Kavetsky Institute are used rather widely in Ukraine [4, 5]. One of the novel developments of the Institute is a xenogeneic antitumor vaccine (KAV), comprising embryonic chicken proteins and cytotoxic protein of *B. subtilis* B-7025. The efficacy of the vaccine was justified in the model experiments in animals and some volunteers [6, 7]. Considering the aforementioned, the authors suggested to explore the combined KAV application with antiviral agents. As an antiviral agent, the preparation Izatizon (active substance – methisazone) was used in the experiments [8]. Izatizon is active against DNA- and RNA-containing viruses. [9]. A wide range of biological activity of izatizon is based on the conformational-labile structure of the molecule methisazone - the main active ingredient of the drug and depends on the properties of the solvent and the microenvironment [10 - 12]. In addition, izatizon has antitumor, anti-inflammatory and antihistamine effects it also has pronounced immunomodulatory properties [13, 14]. The preparation induces the proliferation of T- and B-lymphocytes in systems *in vitro* and *in vivo*, statistically significantly stimulates the synthesis of interleukin-1 by macrophages and interleukin-2 synthesis by

T-lymphocytes in *in vitro* system. The synthesis of these interleukins is an essential component for the activation of antitumor immunity that determines the choice of this preparation for accompanying the therapy with xenogeneic antitumor vaccine [15].

II. Material and Methods

Animals

Sample size: 20 mice.

Antimetastatic effect of combined regimens with xenogeneic antitumor vaccine and Izatizon was explored in the tumor Lewis lung carcinoma growth model (LLC) animals**.** Experiments were conducted in C_{57}B1/6 mice (males, 2.5 months, 20-25 g, bred in the vivarium of R. E. Kavetskyi Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine). The care and handling of the experimental animals have been performed in accordance with generally accepted international rules for conducting experiments in experimental animals. LLC was inoculated intramuscularly into lower limb's thigh (by $0.5x10^6$ cells/animal). .

The following experimental groups were studied (5 mice in group):1т1111111111111111111111111111111111
1 - tumor growth control (TGC) – animals with transplanted LLC tumor;
2 - animals with LLC, receiving preparation Izatizon by 2,5 μl/animal per administration;
3- animals with LLC, receiving xenogeneic antitumor vaccine and preparation Izatizon;
4 - animals with LLC, receiving xenogeneic antitumor vaccine by 300 μl/animal per administration.ь
During the experiment, the dynamics of tumor growth was evaluated.

Vaccine

The treatment of animals was stared on day 15 after tumor transplantation. For vaccine therapy, xenogeneic antitumor vaccine (XAV) was applied, which comprised high-molecular proteins of chicken embryo (0.3 mg/ml of the vaccine) and cytotoxic metabolite of *B. subtilis B-7025* with a molecular mass of 70 kDa (0.3 mg/ml of the vaccine) [16]. The vaccine was administered subcutaneously into animal withers (on days 15, 18, 25, 30, and 34 of the tumor growth) in an amount of 0.3 ml/injection.

Izatizon

Izatizon was produced in the laboratory of the Institute of Molecular Biology and Genetics of the NAS of Ukraine and the Institute of Health Promotion and Rebirth of People of Ukraine according to the Patent of Ukraine No. 1780, 1994. The purity of the final product was confirmed by analytical assays.

The preparation Izatizon, diluted 20-fold by saline, was orally administered 10 times every other day in an amount of 50 μl/animal. The total dose was 500 μl/animal for the entire therapy course.

Evaluation of antimetastatic effect

Antimetastatic action was evaluated on day 37 after LLC transplantation by the number and volume of metastases in the lungs: metastases rate (%); the average number of metastases per one mouse; the average volume of metastases per one mouse. Cytotoxic activity (CTA) of lymphocytes (L) and macrophages (M) was evaluated *in vitro* with the MTT-test [17] on day 37 after tumor transplantation. LLC cells were used as target cells. To determine the level of IgG to the surface antigens of LLC tumor cells ELISA was used [18].

Gelatinase assay

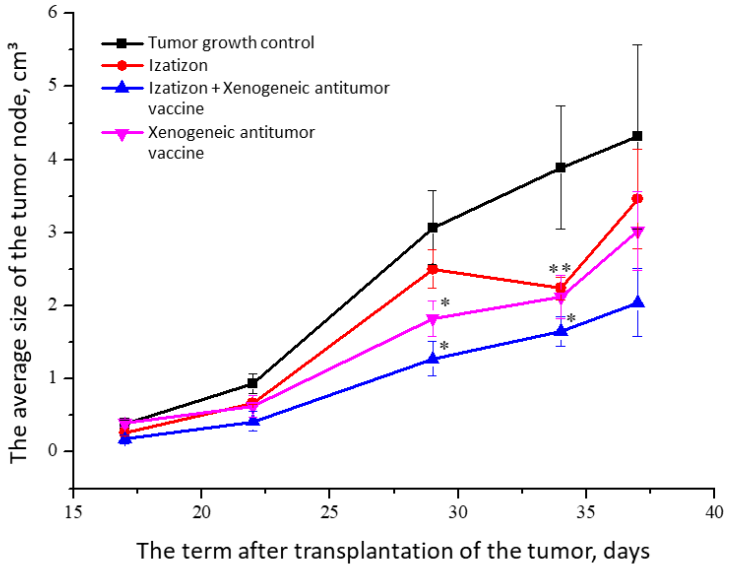
Gelatinase activity was determined by the SDS-electrophoresis method with the addition of gelatine in an amount of 1 mg/ml. Electrophoretic separation of the lysates was performed in the gel, containing 12.5% of acryl amide. Further the gel was incubated in buffer solutions and dyed with coomassie blue R-250 [19]. At that, the zones, containing MMP (matrix metalloproteinase), were developed in a form of undyed bands.

Statistical processing

The significance of the differences was analyzed by Student *t*-test by Microsoft Excel and Microcal Origin software.. The difference were considered as significant if $p < 0.05$.

III. Results

Visual assessment of the graph where the growth dynamics of the tumor nodule determined that Izatizon application in combination with the vaccine led to the maximal delay of tumor nodule growth (Fig. 1). Separate use either vaccine or Izatizon was less effective. On day 34 the difference in tumor nodule size was the following: 3.89±0.84 mm^3 – in tumor growth control; 2.24±0.15 mm^3 – in Izatizon group; 2.12±0.30 $мм^3$ – XAV, and 1.65±0.20 mm^3 ($p \leq 0.05$, compared to TGC) – Izatizon+XAV. At the end of the experiment (day 37 of tumor growth) the average tumor size in the groups was the following: 4.31±1.26 mm^3 – TGC, 3.46±0.68 mm^3 – Izatizon, 3.03±0.54 mm^3 – XAV, 2.05±0.466 mm^3 – Izatizon+XAV.



*p<0.05 compared to control group (TGC) values

Figure no 1 Tumor growth dynamics in the animals with transplanted Lewis lung carcinoma.

Assessing survival (Fig. 2), it was determined that in the group of tumor growth control the survival at the end of the experiment on day 37 was 33%. The survival in other groups was the following: 72% - in Izatizon + XAV" group, 66% - in the XAV group, and 57% in Izatizon group.

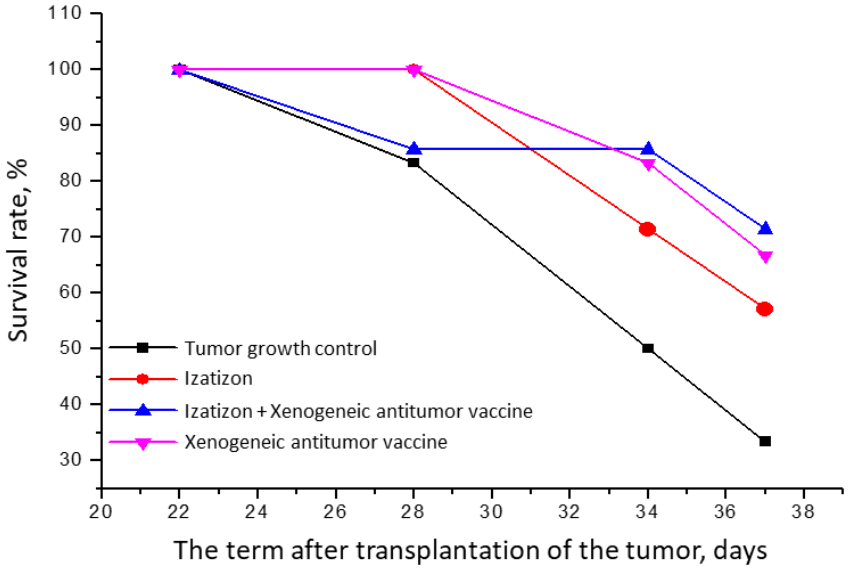


Figure no 2. The survival of animals with transplanted Lewis lung carcinoma.

At the assessment of the level of lung metastatic affection in animals, it was found that the combined use of Izatizon with the vaccine led to a significant inhibition of metastatic potential (number and volume of metastases). Xenovaccine and Izatizon administration in a monotherapy mode was also rather beneficial, although less effective compared with their combined application.

Table no 1. The level of lung metastatic affection in animals with Lewis lung carcinoma

Group	Average metastases volume, mm^3/animal	Average metastases number per animal
Tumor growth control	141.87±62.07	75.5±23.5
Izatizon	28.32±12.43	29.4±11.17
Izatizon + xenogeneic antitumor vaccine	12.36±4.39*	10±3.58*
Vaccine	19.49±17.79	18±17*

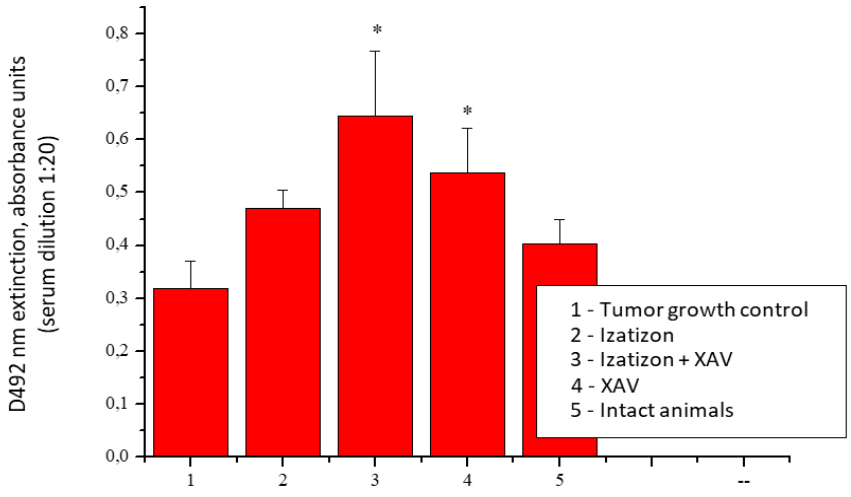
*p<0.05 compared to control grouup values (TGC)

At the assessment of the indicators of average life duration (at the term of euthanasia) (Table 2) it was determined that, among all study groups, this index was the highest in the group receiving the combined administration of Izatizon and vaccine.

Table no 2. The effects of xenogeneic antitumor vaccine and preparation Izatizon on the average life duration of animals with Lewis lung carcinoma

Group	Average life duration, days/animal
Tumor growth control	33.5±1.63
Izatizon	35.43±1.41
Izatizon + xenogeneic antitumor vaccine	**36.71±1.25**
Xenogeneic antitumor vaccine	35±0.82

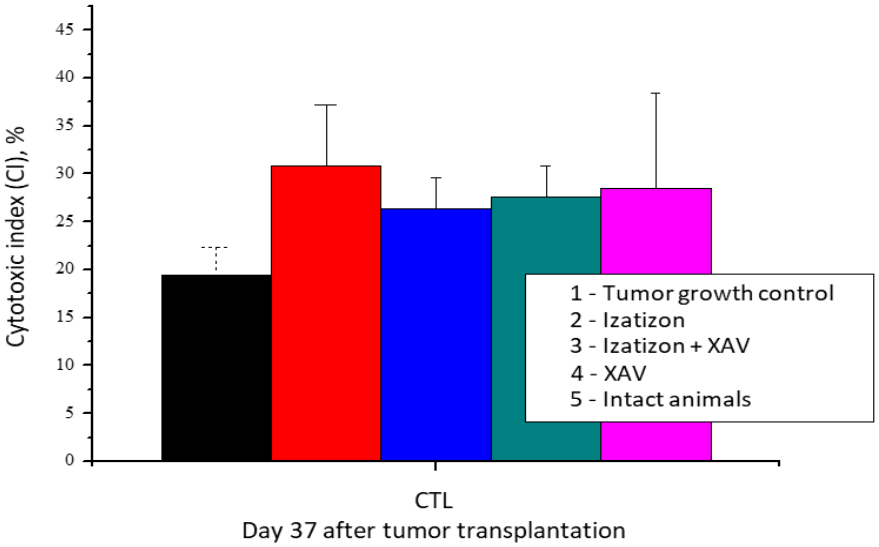
When assessing serum blood levels of immunoglobulins (Fig. 3), it was determined that in the animal groups, gaining a positive therapy effect, the level of class G antibodies was significantly higher than those in the tumor growth control. Specifically, in the group of animals that received a combination of Izatizon with xenovaccine, the level of antibodies was the highest possible and estimated 0.65 ± 0.12 absorbance units (AU) vs 0.32 ± 0.05 AU in the tumor growth control. When using Izatizon and xenovaccine in mono mode, the level of antibodies was slightly lower and estimated 0.47 ± 0.03 AU and 0.54 ± 0.08 AU, respectively.



*p<0.05 compared to control group (TGC) values
Y - D492 nm extinction, absorbance units (serum dilution 1:20(. 1 – Tumor growth control, 2 – Izatizon, 3 – Izatizon + XAV, 4 –XAV, 5 – Intact animals

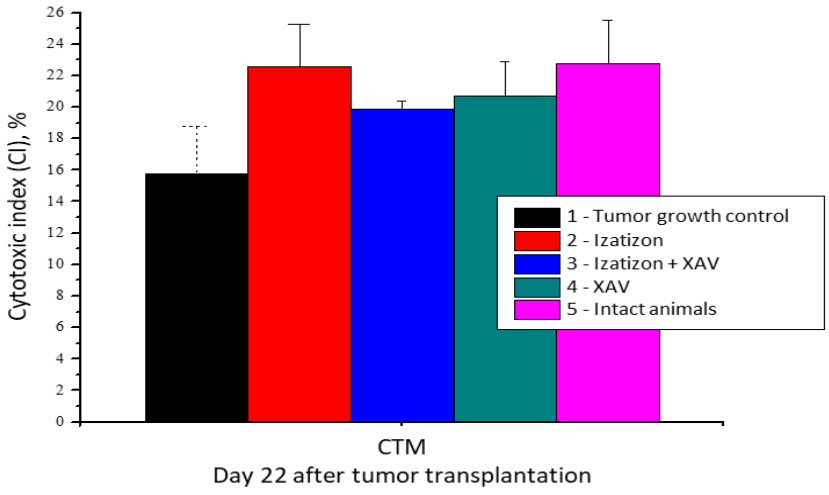
Figure no 3. IgG level in blood serum of animals with Lewis lung carcinoma.

In the study of cytotoxic lymphocyte activity (Fig. 4) it was determined that the statistically reliable differences in the values of all study groups were not seen. Although, in animal groups, receiving antitumor therapy, the cytotoxic activity was substantially higher than the values of tumor growth control (CI=19.47±2.87%). The highest lymphocyte cytotoxicity index was in animal groups that received the preparation Izatizon (CI=30.87±6.29%) and та Izatizon + xenogeneic antitumor vaccine" (CI=27.58±3.23%). In the group of animals that received XAV CI was 26.36±3.32% vs 28.54±9.87% in intact animals. As can be seen, the therapy with study preparations provided with the preservation of lymphocyte cytotoxic activity.



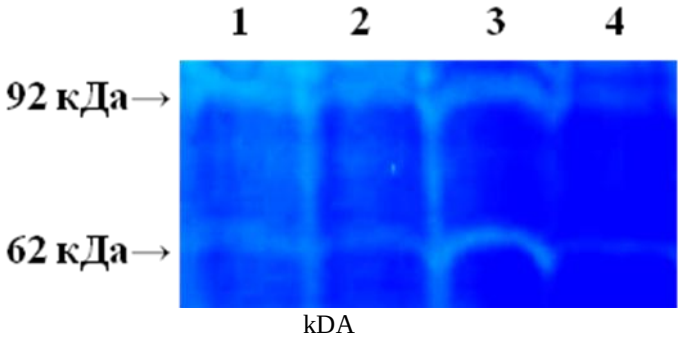
Y - Cytotoxic index (CI), % CTL
Day 37 after tumor transplantation
*p<0.05 compared to control group (TGC) values
Figure no 4. Cytotoxic activity of lymphocytes (CTL).

In the study of macrophage cytotoxic activity (Fig. 5) a dependence that is similar to that observed in the study of cytotoxic activity of lymphocytes, was obtained. Specifically, in the animals that received the therapy the cytotoxicity of macrophages was slightly higher and the CI was 22.57±2.70% in the group Izatizon, 19.87±0.53% in the group Izatizon + XAV" and 20,72 ± 2.16% in the group "TGC". In tumor growth control, the cytotoxic activity of macrophages was 15.79± 2.97% and 22.73 ± 2.80 in intact animals.



CTM
Day 22 after tumor transplantation
Figure no 5. Cytotoxic activity of macrophages.

Lysates were prepared from tumors and tested for gelatinase (metalloproteinase) activity by the zymography method (Fig. 6).



kDA

Figure no 6. Zymography of the lysates, prepared from tumors of animals with transplanted Lewis lung (1- Tumor growth control, 2- Izatizon, 3- XAV, 4- Izatizon+XAV)

When analyzing zymography (Fig. 6), it was determined that in animal groups that received Izatizon the gelatinase levels with a molecular mass of 92 kDa was significantly higher than in other study groups. Gelatinase with molecular mass of 62 kDa was present in the lysates of XAV and Izatizon groups. In the animal groups that received Izatizon with XAV the level of both gelatinases was slightly lower compared to other study groups. One of the attributes of tumor growth is an increase in the level of gelatinase, which is a subfamily of metalloproteinases. Studies of the mechanisms of tumor progression have shown that matrix metalloproteinases (MMPs) play a key role in initiating the processes of angiogenesis, invasion and metastatic spreading [20—23]. These proteinases impact on migration, differentiation, and proliferation of tumor cells. In addition, it was demonstrated that MMPs are involved in the release of deposited growth factors. Given all the above, MMs can be considered an important factor in the aggressiveness of tumor growth. MMP-2 and MMP-9 (gelatinase A/collagenase-2 (72 kDa) and gelatinase B/collagenase-9 (94 kDa), whose genes are located on chromosomes 16q13 and 20q11.2 — q13.1, respectively, have main function that related to the cleavage of neurofibrillary collagen type IV. The recent literature data show that, in fact, these proteinases help tumor growth processes, facilitate tumor invasion and are promising markers of metastasis spreading [24, 25]. Numerous studies have demonstrated that high levels of expression and activity of gelatinases were observed in tumors of different locations, such as lung cancer, pancreatic cancer, bladder cancer, kidney cancer, ovarian cancer and others [26 - 28].

The work was implemented in the framework of the project "Study of the functional plasticity of macrophages under the conditions of cancer chemotherapy and biotherapy" 2019-2023.

IV. Conclusions

1. It was found that the combined application of xenogeneic antitumor vaccine with preparation Izatizon in the model of Lewis lung carcinoma let to the delay of the development of the primary tumor nodule and almost ten times reduced the number of metastatic lesions in the lungs of experimental animals.
2. Antitumor effects of the combined application of xenogeneic antitumor vaccine with preparation Izatizon were observed due to the increased synthesis of IgG to tumor proteins and preservation of cytotoxic macrophage and lymphocyte activity.
3. In the lysates prepared from the tumors of treated animals, a substantial decrease in the gelatinase number was seen, suggesting a reduction in its proliferative activity.
4. The experimental data demonstrate the need for antiviral preparation application on the background of tumor growth at combined application with other biotherapeutic agents.

References

[1]. Fedorenko ZP, Mykhalovych YuJ, Gulak LO et al. Cancer in Ukraine, 2013-2014. Bull. of the National Cancer Register 2015; (16): 1-6 (In Ukrainian).
[2]. Hu J, Ludgate L (2007). HIV-HBV and HIV-HCV coinfection and liver cancer development. *Cancer Treat. Res.* 133: 241–52.
[3]. Bellon M, Nicot C (2007). «Telomerase: a crucial player in HTLV-I-induced human T-cell leukemia». *Cancer genomics & proteomics* 4 (1): 21–5.
[4]. Potebnya GP, Khutornoi SV, Didenko GV. (2002) Evaluation of effectiveness of antitumor vaccines obtained with the use of metabolic products of *Bacillus subtilis AB-56*. Exp Oncol; 24(3): 225-8.
[5]. Lisovenko GS, Potebnya GP, Kolesnik EA, Kikot VV. (2002) Application of tumor marker antigens on immunotherapy of patients with colorectal cancer. Exp Oncol; 24(4): 258-64.
[6]. Kuzmenko O.P., Didenko G.V., Potebnya G.P. Comparative evaluation of antitumor efficacy of allogeneic and xenogeneic vaccines modified with metabolites of B. subtilis B-7025 with a molecular weight of 18 and 70 kDa in the experiment. Oncology, No. 4, 2014., p.82-86 (In Ukrainian).

[7]. Utility model patent No. 77647 (UA). The method antitumor vaccine preparation / Potebnya GP, Didenko GV, Kuzmentko OP, Shpak EG, Lisovenko GC, Cheremshenko YK, Simchych TV, Chekhun VF (Ukraine); application No. u2012 08724 filed 16.07.2012; Publication on 25.02.2013, Bull. No. 4 (In Ukrainian).
[8]. Patent of Ukraine No. 1786 Antiviral preparation izatizon / Potopalskyi A.I., Loziuk L.V. -29.10.93 (25.10.95. Bull. No. 3 (In Ukrainian).
[9]. Bolsunova Ol'ha I., Zaika Leonid A., Potopalsky Anatoliy I., Voznyuk Anna V. Izatizon, as an izatin-thiosemicarbazone derivative, has antiviral, anti-tumor actions and no side effects // International Journal of Pharmaceutical Science Invention -2017 -V.6. -Is.5. – P.7-9 (9)].
[10]. A.L. Potyagailo, O.I. Bolsunova, L.A. Zaika, A.I. Potopalskyi, D.M. Govorun. Conformational analysis of the metisazone molecule: results of semi-empirical quantum-chemical calculations //Biopolymers and cells, 2003, vol. 19, no. 3, pp. 292-294. (In Ukrainian).
[11]. Bolsunova OI, Potyagailo AN, Govorun DM, Zaika LA, Kharchenko VM Immunobiological activity of izatizon and its relationship with the structural and conformational features of the metisason molecule. - Proceedings of the International Scientific and Practical Forum "Fundamentals of Molecular and Genetic Rehabilitation of Man and the Environment" Kyiv, - 2005.- P. 19- 21 (In Ukrainian).
[12]. Ol'ha I. Bolsunova, Ol'ha O. Brovarets', Dmytro M. Hovorun, Leonid A. Zaika, Anatoliy I. Potopalsky Quantum chemical analysis of structural and conformational properties of methisazone and prototropic tautomerism of isatin International Reviews of Biophysical Chamistry (I.RE.DI.C.) Published 31 October 2011.
[13]. Zaika L. Bolsunova O., Potopalsky A.,Didenko G, Kruts O. Antitumor activity of antiviral substance isatizon //
[14]. Experimental Oncology, 2019, Vol.41, No3 (Materials of the II International Scientific Conference "Tumor and Host Novel Aspects of Old Problem", Nov 21, 2019).
[15]. http://iepor.org.ua/conferences/abstract-2019-11-21-22.html
[16]. S. Rybalk1, D. Starosyla, L. Zaika, O. Bolsunova, A. Potopalskyi, B. Zadorozhniy, M. Arkhipova, O. Deriabin, M. Zavelevich Izatizon Efficacy against Coronavirus Infection in Model of Transmissive Gastroenteritis Virus of Pigs IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS) e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 5 Ser. III (Sep. –Oct. 2021), PP 36-39 www.Iosrjournals.Org.
[17]. G.V. Didenko, L.V. Sorokina, Eu.G. Shpak, O.A. Golub, O.G. Lysenko, G.P. Potebnya1 and A.G. Mamalis The application of fullerene C60 for the modification of an anticancer vaccine based on metabolism products of *Bacillus subtilis* 7025 *Journal of Biological Physics and Chemistry* 11 (2011) 30–35 © 2011.
[18]. Ohno M., Abe T. Rapid colorimetric assay for the quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6). J Immunol Meth. 1991; 145: 199–203.
[19]. Didenko G.V. Modification of an antitumor autovaccine based on metabolic products of *B. Subtilis 7025* using sorbents and auto-macrophages / G.V. Didenko, O.S.Dvorshchenko, G.S.Lisovenko // Experimental oncology – 2003. –No. 2. – P. 116-118 (In Russian).
[20]. Oliver G. W., Stettler-Stevenson W. G., Kleiner D. E. 1999. Zymography, casein zymography and reverse zymography: activity assays for proteases and their inhibitors. In: Handbook of proteolyitic enzymes. San Diego: Acad. Press. 61—76.
[21]. Metalloproteinases as regulators of angiogenesis in malignant neoplasias / Spirina L.V, Kondakova I.V., Lishko E.V., Kakurina G.V., Shishkin D.A. // Sib. Oncol. Journ. — 2007. — V. 21, No. 1. — P. 67—71 (In Russian).
[22]. Curran S., Murray G. I. Matrix metalloproteinases: molecular aspects of their in tumour invasion and metastasis // Eur. J. Cancer. — 2000. — Vol. 36, N 13. — P. 1621—1630.
[23]. Roles of matrix metalloproteinases in tumor metastasis and angiogenesis / Yoon S. O., Park S. J., Yun C. H., Chang A. S. // J. Biochem. Mol. Biol. — 2003. — Vol. 36. — P. 128—137.
[24]. Biological role and regulation of matrix metalloproteinases in cancer / Davidson B., Reich R., Risberg B., Nesland J. M. //Arch. Pathol. — 2002. — V. 64, No. 3. — P. 47—53 (In Russian).
[25]. Visse R., Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function and biochemistry // Circul. Res. — 2003. — Vol. 92. — P. 827—839.
[26]. Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 in colorectal carcinoma invasion and metastasis / Li B. H., Zhao P., Liu S. Z., Yu Y. M., Han M., Wen J. K. // World J. Gastroenterol. — 2005. — Vol. 28, N 20. — P. 3046—3050.
[27]. Delektorskaya V.V. Molecular biological markers of metastasis and prognosis in colon cancer: Doctoral thesis. — M., 2007. — 247 p. (In Russian).
[28]. Prognostic significance of proteinases in patients with squamous cell carcinomas of the head and neck / Klisho E.V., Kondakova I.V., Choinzonov E.L., Vasilieva O.S. // Bull. SB RAMS. — 2005. — No. 2. — P. 82—91 (In Russian).
[29]. Turpeenniemi-Hujanen T. Gelatinases (MMP-2 and -9) and their natural inhibitors as prognostic indicators in solid cancers // Biochimie.— 2005. — Vol. 87, N 3—4. — P. 287—297.
[30]. Matrix metalloproteinases 2 and 9 in oral squamous cell carcinomas:manifestation and localization of their activity / Kato K., Hara A.,Kuno T., Kitaori N., Huilan Z., Mori H., Toida M., Shibata T. // J. CancerRes. Clin. Oncol. — 2005. — Vol. 131, N 6. — P. 340—346.

Cruts O.O, et. al. "Promising Use of the Combination of Xenogeneic Antitumor Vaccine and Preparation Izatizon in Malignant Tumors." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 17(4), (2022): pp. 09-15.