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ELEVATION OF EFFICACY OF CANCER VACCINE COMBINED WITH INTERFERON AND INDUCER OF ENDOGENEOUS INTERFERON SYNTHESIS AMIXIN

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Aim: **To study *in vivo* efficacy of combined administration of cancer vaccine (CV), interferon (IFN) and inducer of endogenous IFN — amixin.** ***Materials and Methods*****: Sarcoma-37 cells were transplanted to female Balb/c mice. For the treatment, CV prepared from sarcoma-37 cells with the use of cytotoxic lectines from *B. subtilis B-7025*, murine IFN and amixin or their combinations were used. IFN production, content of circulating immune complexes and level of specific IgG antibodies in blood serum were determined by standard immunologic methods.** ***Results:*** **Using solid form of sarcoma-37 it has been shown that introduction of IFN and amixin significantly elevated efficacy of vaccine therapy, in particular index of tumor growth inhibition reach 89.2% and 81.7%. Upon combined use of CV and IFN or CV and amixin (25 mg/kg) respectively. Significant prolongation of average life span of the animals treated with CV and IFN or CV and amixin (25 mg/kg) has been registered (up to 92.7 ± 10.4 and 95.0 ± 6.2 days respectively, *vs* 46.8 ± 1.5 days for control animals).** ***Conclusion:*** **Obtained results have shown expediency of the development of schemes for combined introduction of CV with exogenous IFN, and with inducer of endogenous IFN (amixin) for elevation of efficacy of vaccine therapy.**

Key Words: **cancer vaccine, interferon, amixin, experimental tumor, immunologic index.**

Unsatisfactory results of traditional methods of cancer therapy determine the need for search of new means elevating anticancer resistance of organism. Activation of the mechanisms of immune surveillance is an important way to solve the task. Presently the development of immunotherapeutical approaches, in particular, cancer vaccines (CV) is considered as the most promising approach. Analysis of modern data evidence on high prophylactic and therapeutic efficacy of combined use of specific CV with adjuvants of different origin [1, 2], including various cytokines able to stimulate immune response [3, 4].

Interferon (IFN) was among the first cytokines successfully applied in clinical practice for activation of unspecific resistance upon viral infections and immunodeficiency state [5]. IFN as natural factor of antitumor defense is one of key modulators of immune response, and influences the processes of antigen recognition, differentiation, recruitment and functional activity of immunocompetent cells. Also IFN has direct influence on tumor growth and differentiation and act as apoptosis inducer [6, 7].

Effects of IFN related to elevation of tumor immunogenecity and its altered sensitivity to cytotoxic and differentiation-stimulating action of T-lymphocytes are of particular interest [7], as well as the role of IFN at combination with other biological agents. The special attention is focused on the study of effects of IFN combined with active specific immunization [8–10].

That's why the search for compounds able to activate synthesis of endogeneous IFN — so called IFN inducers, is of great importance [11, 12]. Amixin (tyrolon, 2,7-bis [2-(diethyamino) etoxy] fluorenon-9 dihydrochloride) is a peroral low molecular weight inducer of endogenous IFN that may be used for prophylaxis and therapy of a number of diseases. Activity of this preparation is related to its immunomodulating properties, ability to normalize impaired immune reaction of organism, renew a balance of important elements of immune system [13–15]. The data on significant antitumor activity of amixin were obtained in experimental studies using model tumors, and in clinical trials for melanoma, breast cancer, renal-cell cancer [13]. Moreover, it has been shown that combined use of amixin and anticancer drugs promoted growth inhibition of experimental tumors and decreased metastasis rate [16].

In our earlier studies it has been shown that introduction of IFN to CV significantly elevated efficacy of vaccine therapy of Lewis lung carcinoma [17]. The aim of present research was to evaluate possible benefits of the use of amixin for elevation of CV efficacy, and to compare the obtained results with these for IFN.

MATERIALS AND METHODS

In the work, female Balb/c mice weighting 18–20 g, 2.0–2.5 months old bred in the vivarium of IEPOR NASU (Kyiv, Ukraine) were used. As tumor model, solid form of sarcoma-37 was used.

Experimental animals (n = 8 per group) were routinely transplanted intramuscularly with 5.0 x 10^5 tumor cells, and received the treatment according to the scheme of experiment (Table 1). CV was prepared from sarcoma-37 cells and cytotoxic lectine (CL) from *B. subtilis B-7025* according to the method [18, 19]. CV was administered 5 times at equal doses (0.3 ml) subcutaneously on days 1, 4, 8, 12 and 15 after tumor transplantation.

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Abbreviations used: ALS – average life span; CIC – circulating immune complex; CV – cancer vaccine; IFN – interferon; TGI – tumor growth inhibition.

Experimental protocols and procedures were aproved by the Ethical Committee of IEPOR.

Table 1. Therapeutic experiment scheme for a study of efficacy of combined use of CV and IFN or CV and amixin in sarcoma-37-bearing mice

Group	Treatment	Scheme of administration	
		CV	IFN or amixin
1	CV	By 0.3 ml; s/c; 5-times; at days 1, 4, 8, 12 and 15	–
2	IFN	–	By 1000 U., 5-times; i/p; at days 1,3,7, 11 and 14
3	CV + IFN	By 0.3 ml; s/c; 5-times; at days 1, 4, 8, 12 and 15	By 1000 U, 5- times; i/p; at days 1,3,7, 11 and 14
4	Amixin (10 mg/kg)	–	By 10 mg/kg in 0.5 ml PS; *per os*, 2 h prior to CV
5	CV + amixin (10 mg/kg)	By 0.3 ml, s/c; 5-times; at days 1, 4, 8, 12 and 15	By 10 mg/kg in 0.5 ml PS; *per os*, 2 h prior to CV
6	Amixin (25 mg/kg)	–	By 10 mg/kg in 0.5 ml PS; *per os*, 2 h prior to CV
7	CV + amixin (25 mg/kg)	By 0.3 ml, s/c; 5-times; at days 1, 4, 8, 12 and 15	By 25 mg/kg in 0.5 ml PS; *per os*, 2 h prior to CV
8	Control of transplantation	–	–

Preparation of murine IFN kindly gifted by Dr. Kudryavets was administered 5 times intraperitoneally by 1000 U in 0.5 ml physiologic solution (PS) 24 h prior to CV introduction. Substance of amixin was supplied by OSS "Interkhim", preparation was administered p.o. 5 times at the doses of 0 mg/kg or 25 mg/kg in 0.5 ml, 2 h prior to CV administration. The animals from control group received PS in equal manner and time points.

Efficacy of treatment was evaluated by common tumor growth characteristics, survival and average life span (ALS) of control and experimental animals.

Immunological studies were performed at days 14, 30 and 40 after tumor transplantation, and include determination of the content of circulating immune complexes (CIC) isolated by precipitation with PEG-6000, and analysis of the level of specific IgG antibodies in blood serum by immunoenzyme reaction [20]. Production of IFN was evaluated by titration of blood serum for its ability to inhibit cytopathogenic action of vesicular stomatitis virus [21].

Significance of the differences between the parameters was evaluated by Student's *t*-criterium [22]. Calculations were done using Microsoft Excel program.

RESULTS AND DISCUSSION

Analysis of growth dynamics of sarcoma-37 in the groups of animals treated with studied preparation has demonstrated its significant inhibition (Fig. 1). For example, in animals that received IFN or amixin at monoregimen, tumor growth inhibition (TGI) index was in a range 65–75%, but the difference between experimental groups was insignificant ($p > 0.05$) (Fig. 1, *a*). Respective index in mice treated with CV was 81.2% (Fig. 1, *b*).

Upon application of combined scheme of introduction of studied preparations, the best results were observed in mice that received CV and IFN: TGI was equal to 89.2%. Combined introduction of CV with amixin was also effective, if amixin was introduced at the dose of 25 mg/kg: TGI was 81.7%. The results of combined use of CV with 10 mg/kg amixin were more worse then in the case of separate administration of CV or amixin (TGI = 53.8%, 81.2% and 66.5%, respectively; $p < 0.05$). It seems that low-dose amixin caused even antagonistic effect in its combination with CV. The mechanisms of such effect should be clarified.

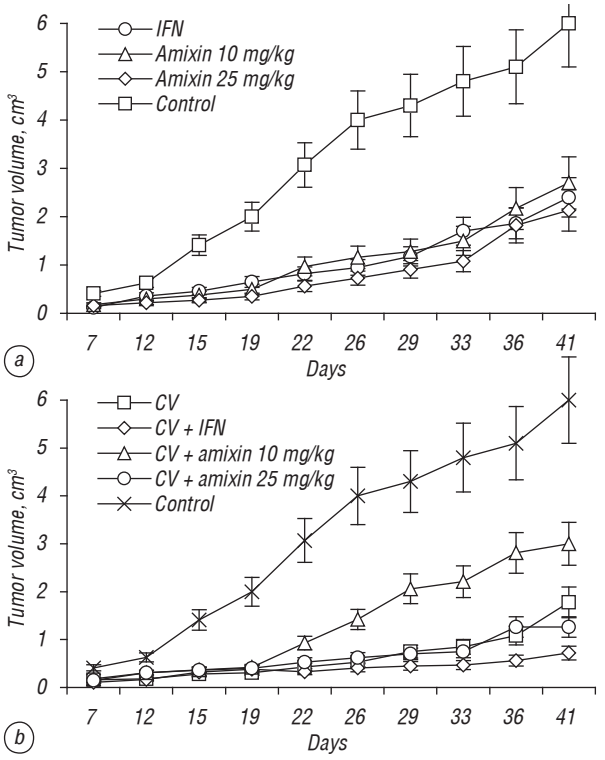

Fig. 1. Dynamics of tumor growth in sarcoma-37-bearing mice treated with IFN or amixin at monoregimen (*a*) or in combination with CV (*b*)

The studied preparations possess different effect on efficacy of sarcoma-37 transplantation (Fig. 2, *a*).

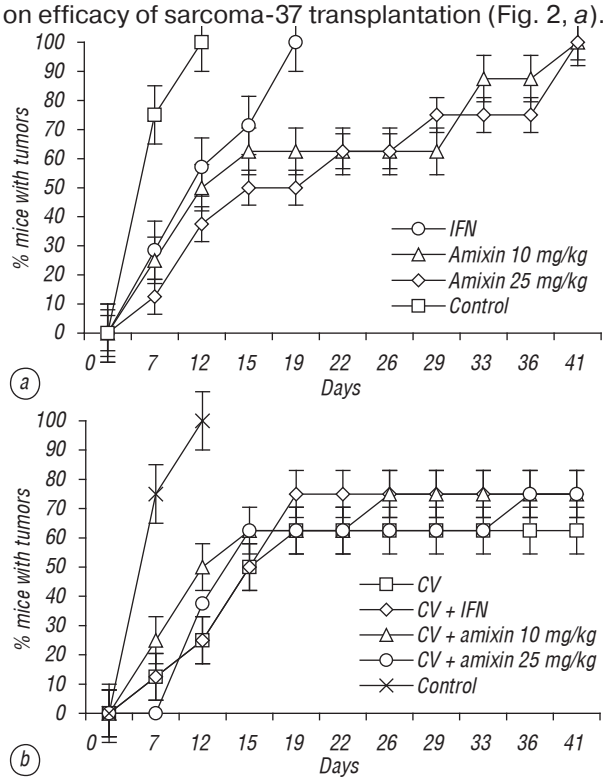

Fig. 2. Efficacy of sarcoma-37 cells transplantation into animals treated with IFN or amixin at monoregimen (*a*) or in combination with CV (*b*)

At day 12, all animals from control group develop tumors, but introduction of IFN or amixin at the doses of 25 and 10 mg/kg caused retardation of this process (at the day 12 indexes of transplantation were 57.1; 50.0 and 37.5%, respectively), however later the tumors developed in all animals. In particular, in mice treated with amixin at both doses, the tumors developed at the

day 41. Introduction of CV resulted in decreased transplantation efficacy in all experimental groups (Fig. 2, *b*). In CV-treated mice this index decreased to 62.5%, and in animals treated with combination of CV with IFN or amixin transplantation efficacy was 75%.

Animal survival rate (Fig. 3) was elevated in all experimental groups: while in control group all animals die before day 54, in IFN-treated mice this index was 71 days, and only 62.5% animals died at day 84, if both doses of amixin were used. The highest survival has been recorded in mice treated with combination of CV with IFN or amixin (25 mg/kg) — at day 84, only 25% animals dies in each group, and such index was significantly higher compared to the use of CV at monoregimen or with amixin at low dose (62.5% in each case).

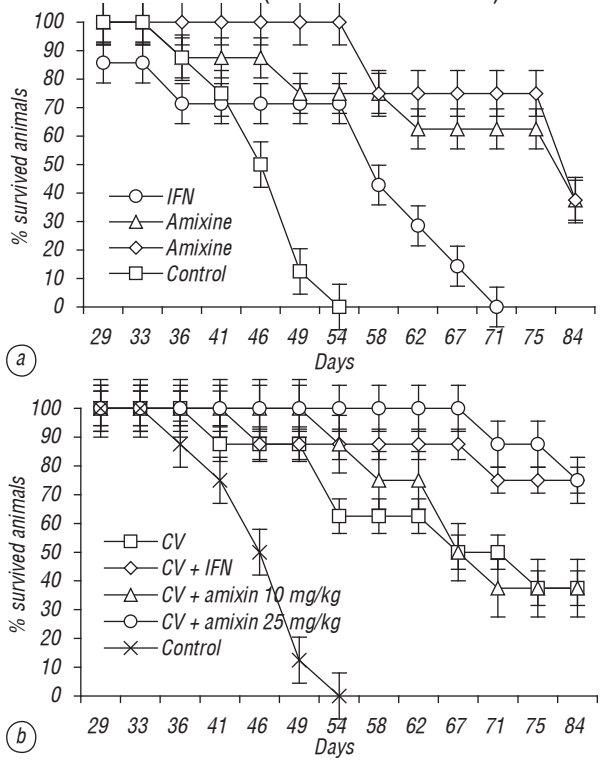

Fig. 3. Survival of sarcoma-37-bearing animals treated with IFN or amixin at monoregimen (*a*) or in combination with CV (*b*)

Analysis of ALS (Table 2) was in agreement with these observations: the best results were registered exactly upon combined use of CV and IFN or CV with 25mg/kg amixin (ALS indexes were 92.7 ± 10.4 and 95.0 ± 6.2 days *vs* 46.8 ± 1.5 days in control group, $p < 0.05$). These indexes were significantly higher than these for separate use of each preparation (ALS indexes in mice treated with CV, IFN or 25 mg/kg amixin at monoregimen were 79.3 ± 10.9, 64.7 ± 10.1, and 79.0 ± 5.9 days, respectively). So, abovementioned data allow state elevation of efficacy of CV upon its combined use with IFN or amixin. Decreased amixin dose (10 mg/kg) introduced into combined scheme did not cause such effect: ALS did not differ significantly from that for separate introduction of (77.0 ± 7.8 *vs* 77.0 ± 10.1 days, $p > 0.05$), but was significantly higher than ALS in control group ($p < 0.05$).

Determination of IFN titers in blood serum of experimental animals did not reveal the peculiarities explaining clearly the different efficacy of used schemes of IFN or amixin administration (Table 3). Compared to the control group, in all experimental groups there was observed elevation of IFN titers at day 14 and especially at day 30 of tumor growth, and its decrease at day 40. In control tumor bearing animals these indexes were 1 : 64; 1 : 64; 1 : 16 respectively.

Table 2. Average life span of sarcoma-37-bearing mice treated with IFN or amixin at monoregimen or in combination with CV

Group	Treatment	Number of animals	ALS		
			X ± m	t	MI%
1	CV	8	79.3 ± 10.9	2.93	69.4
2	IFN	8	64.7 ± 10.1	1.73	38.2
3	CV + IFN	8	92.7 ± 10.4	4.31	98.0
4	CV + amixin 10 mg/kg	8	77.0 ± 7.8	3.79	64.5
5	Amixin 10 mg/kg	8	77.0 ± 10.1	3.04	66.8
6	CV + amixin 25 mg/kg	8	95.0 ± 6.2	7.56	102.9
7	Amixin 25 mg/kg	8	79.0 ± 5.9	5.32	68.8
8	Control	8	46.8 ± 1.5		

Table 3. IFN titers in blood serum of sarcoma-37-bearing mice treated with IFN or amixin at monoregimen or in combination with CV

Group	Treatment	Days after tumor transplantation		
		14	30	40
1	CV	1 : 128	1 : 128	1 : 16
2	IFN	1 : 32	1 : 16	1 : 16
3	CV + IFN	1 : 32	1 : 64	1 : 64
4	CV + amixin 10 mg/kg	1 : 64	1 : 128	1 : 32
5	Amixin 10 mg/kg	1 : 64	1 : 256	1 : 32
6	CV + amixin 25 mg/kg	1 : 64	1 : 256	1 : 32
7	Amixin 25 mg/kg	1 : 64	1 : 128	1 : 16
8	Control	1 : 64	1 : 64	1 : 16
9	Intact animals	1 : 16	1 : 16	1 : 16

Using immunoenzyme assay (IEA) we have determined the level of sarcoma-37 antigens in blood serum of experimental animals (Fig. 4). The results evidence on significant increase of this index in control animals during all terms of observation, even at late stages of tumor growth, however, in mice treated with IFN or CV at monoregimen or in combination, the level of antitumor antibodies was higher, pointing on immunostimulating action of the preparations. The use of amixin at the dose of 25 mg/ml in both schemes significantly decreased this index compared to the control. In contrary, introduction of low-dose amixin resulted in the level of antibodies at days 30–40 of tumor growth close to control values, while its combined use with CV also leads to sharp decrease of IgG levels at the late terms of tumor development.

The study of content of medium-molecular weight CIC in blood of control tumor-bearing group has shown its elevation compared to intact animals, as well as its drastic increase (up to 0.777 ou.) at late stages of tumor growth (Fig. 5). Upon administration of IFN, the dynamics of CIC content seems to be similar to that of intact animals, except its low value (0.183 ou.) at day 14 of tumor growth. At day 14 similarly low level of CIC has been detected also in the case of CV administration (at monoregimen or in combination with IFN), but in this case at day 40, sharp decrease of CIC level was observed after elevation of this index at day 30 characteristic for the majority of experimental groups.

In contrary, introduction of amixin was accompanied with elevation of CIC level at day 14 of tumor growth (0.520–0.540 ou), close to this index in the group of tumor-bearing control animals. However, at day 40, CIC level has been decreasing fluently (25 mg/ml dose), or sharply (10 mg/ml dose). Combined use of amixin and CV also leads to sharp decrease of CIC content in blood serum

at respective time point. It is necessary to note that at late terms after tumor transplantation (day 40) in mice treated with CV or amixin by different schemes, sharp decrease of this index has been registered compared to control animals; according to the data of literature, such effect point on favorable prognosis of the disease [23, 24]. In our study it was accompanied by significant tumor growth inhibition and elevation of life span of vaccinated animals.

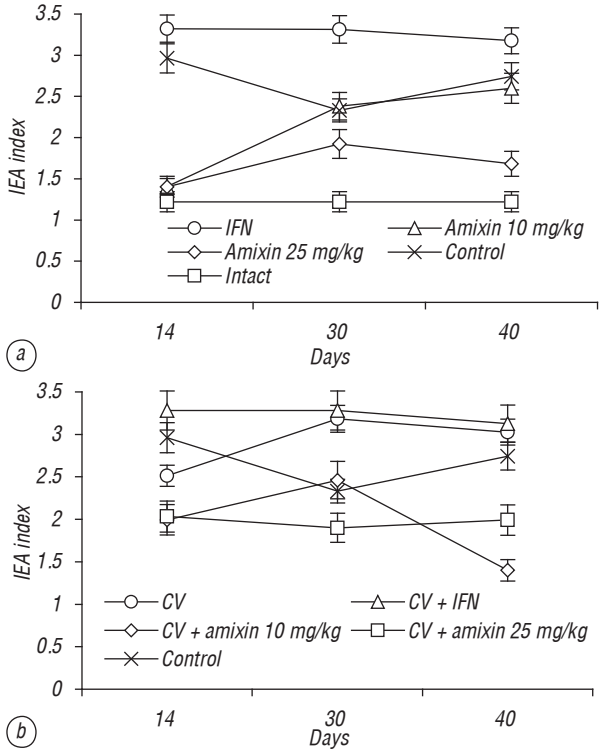

Fig. 4. IgG levels in blood serum of sarcoma-37-bearing mice treated with IFN or amixin at monoregimen (*a*) or in combination with CV (*b*)

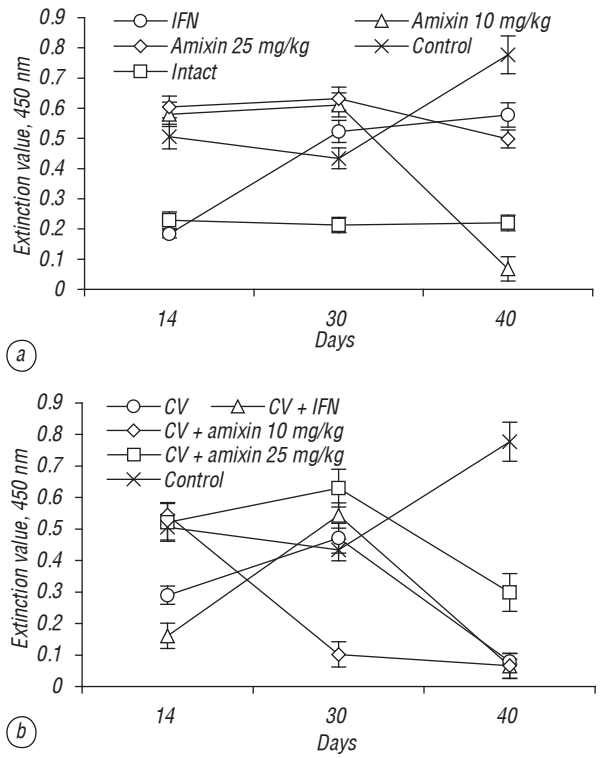

Fig. 5. CIC levels in sarcoma-37-bearing mice treated with IFN or amixin at monoregimen (*a*) or in combination with CV (*b*)

In conclusion, above mentioned results have demonstrated that inducer of synthesis of endogenous IFN amixin at a dose of 25 mg/kg as well as exogenous IFN at a dose of 1000 U are able to elevate equally anticancer action of the vaccine. Our data evidence on possibility for improvement of efficacy of cancer vaccine via development of combined schemes for its use.

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ПОВЫШЕНИЕ ЭФФЕКТИВНОСТИ ПРОТИВООПУХОЛЕВОЙ ВАКЦИНЫ ИНТЕФЕРОНОМ И ИНДУКТОРОМ СИНТЕЗА ЭНДОГЕННОГО ИНТЕРФЕРОНА — АМИКСИНОМ

Цель: **изучить в эксперименте эффективность комбинированной схемы введения противоопухолевой вакцины (CV) с интерфероном (ИФН) и индуктором эндогенного ИФН — амиксином.** ***Материалы и методы*****: саркому-37 трансплантировали мышам-самкам Balb/c. Для лечения использовали CV, приготовленную из клеток саркомы-37 с помощью цитотоксических лектинов *B. subtilis B-7025*, мышиный ИФН (1000 ед.) и амиксин (10 и 25 мг/кг). Иммунологические исследования включали определение в сыворотке крови титров ИФН, количества циркулирующих иммунных комплексов и уровня специфических противоопухолевых IgG-антител.** ***Результаты:*** **на модели солидной формы саркомы-37 показано, что применение ИФН и амиксина достоверно способствует повышению результатов вакцинотерапии, а именно, при комбинированном использовании CV и ИФН индекс торможения опухолевого роста (ИТО) достигал 89,2%; при сочетании CV и амиксина (25 мг/кг) ИТО составил 81,7%. Зарегистрировано существенное увеличение средней продолжительности жизни животных, получивших CV с ИФН или амиксином (25 мг/кг), до 92,7 ± 10,4 и 95,0 ± 6,2 сут соответственно, по сравнению с такой контрольных мышей (46,8 ± 1,5 сут, $p < 0,05$).** ***Выводы:*** **полученные результаты свидетельствуют о перспективности разработки комбинированных схем введения CV как с препаратом экзогенного ИФН, так и с индуктором эндогенного ИФН (амиксин), что позволяет повысить эффективность вакцинотерапии.**

Ключевые слова: **противоопухолевая вакцина, интерферон, амиксин, экспериментальная опухоль, показатели иммунитета.**

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