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Xenovaccinotherapy for Cancer

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1. Introduction

Up to date, a systemic treatment of cancer is based mainly on the use of chemotherapy. However, in the majority of cases, chemotherapy is not a radical treatment. In initially identified tumors there already exist cells that are resistant to toxic drug action, due to their biochemical properties. Furthermore, the proportion of such cells is progressively increased throughout the treatment period because they receive selective growth advantages over the cytotoxic drug-susceptible cells. It should also be noted that cytotoxic action of antineoplastic drugs is not selective: the drugs affect not only tumor, but also normal cells. Hence, there may be serious side effects of chemotherapy, which, by themselves, may be life-dangerous and frequently requiring further medical interventions. An appearance of the drugs with selective cytotoxic activity seems improbable in the near future because the vital biochemical processes in tumor and normal cells are similar in their basis.

Nevertheless, tumor cells are distinguished from normal ones by quantitative and qualitative expression on their surfaces of potentially immunogenic structures (antigens). It is generally accepted that the immune responses induced by these structures can cause destruction of tumor cells, and that reactivity of the immune system can define an outcome of disease. All of the tumor-associated antigens (TAA) can be divided into two groups: the first one involves the differentiation antigens that can be expressed in not only tumor, but also normal cells, whereas the second one comprises of the products of mutated or viral genes, which can be expressed exclusively in malignant cells. The vast majority of TAA belongs to the first group. These TAA can be expressed in a variety of tumors, due to commonality in the intracellular mechanisms involved in malignizeition of various types of cells. There is considerable interest in developing therapeutic vaccines for cancer, as they hold the promise of delaying or preventing cancer recurrence, particularly in early-stage disease patients. However, there exists a general problem with cancer vaccine application, because most of the TAA are represented by self, nonmutated proteins which are poorly immunogenic for the immune system [reviewed in 1]. Hence, overcoming the immune tolerance toward TAA is indeed a key task of cancer immunotherapy. The use of vaccines based on xenogenic TAA seems to be a promising approach to resolving this problem. Since TAA are typically evolutionarily conservative molecules, there is a strong homology between human and animal TAA. On the other hand, small interspecific structural differences of TAA can be advantageously used in constructing cancer vaccines because xenoantigens may potentially represent an "altered self", with sufficient differences from self-antigens to render them immunogenic, but with sufficient similarities to allow reactive T cells to maintain recognition of self [1].

2. Xenovaccinotherapy in animal models

The majority of studies concerning xenogenic vaccines have been carried out on animals with melanoma, the tumor that expresses a whole number of potentially immunogenic antigens. There is compelling evidence that xenogenic melanoma- associated antigens are much more effective in inducing antitumor immune responses in mice than are their murine analogs. For example, multiple immunizations of mice with human glycoproteins gp75 and gp-100 were reported [2-6] to be effective in preventing the growth of the syngeneic melanoma cells expressing the appropriate mouse analogs [6]. Interestingly, the murine gp75 being initially non-immunogenic became immunogenic in mice when it was administered in the form expressed on a membrane of insect cells [7]. This suggests that the membrane-bound xenoantigens that are not related to tumorogenesis are capable of contributing to self TAA-driven immune responses. The related melanosomal antigens appear to differ in immune response patterns which they induce. For example, the DNA vaccination of mice with human tirosinase-related protein-1 (TRP-1/gp75) induced antibody- mediated responses and autoimmune depigmentation, whereas the DNA vaccination with human TRP-2 resulted in the generation of tumor-specific CD8+ cytotoxic T lymphocytes (CTL) and failed to elicit depigmentaion [8].

As surgery is essential for any melanoma treatment, experiments have been performed to evaluate antitumor effects of xenovaccination in the postoperative period. It was shown that the postoperative DNA vaccination of mice with human TRP-2 could prevent the development of melanoma metastasis in the lungs [4]. These data suggest that xenovaccinotherapy can be the most effective when applied in addition to surgical treatment.

The polyantigenic vaccination has apparent advantages over the monoantigenic one in achieving clinically relevant antitumor responses, thank to its ability to simultaneously induce immune reactions directed against different TAA. We demonstrated that the survival in the melanoma-bearing mice vaccinated with destroyed human melanoma cells was noticeably superior than that in the control mice immunized with murine tumor cells. Surprisingly, the antitumor effect in this experimental model was associated with the rise of serum interleukin (IL)-4, but not interferon (IFN)-γ [9].

As already mentioned, the differentiation antigens are expressed not only in tumor but also in normal cells. This raises the possibility of obtaining such antigens from normal tissues in which they are highly expressed. Placenta is well known to express a whole range of differentiation antigens, including those shared by different tumors including melanoma [10]. Therefore, placental tissue, could be a suitable source of the xenoantigens applicable for breaking the immunological tolerance to a number of TAA. In fact, the mice that received the soluble proteins derived from the porcine placenta as a vaccine, demonstrated the immune protection from melanoma where both CD4+ and CD8+ T lymphocytes were involved [10].

The xenovaccination aimed at breaking the tolerance to a melanosomal antigen gp 100 has been applied in the treatment of 34 melanoma dogs [11]. Canine melanoma cells 17CM98 transfected with a DNA fragment encoding human gp 100 were used as a vaccine. With such vaccinotherapy, a complete or partial response was achieved in 17% animals, and disease stabilization with a duration of not shorter than 6 weeks was recorded in 35% vaccinated dogs. The clinical responses correlated with delayed-type hypersensitivity (DTH) skin reactivity to vaccinal antigens but was independent both of the functional activity of

vaccine-specific CTL and of the serum level of antivaccinal antibodies [11]. In this study the animals that responded to vaccination, survived significantly longer compared to those which did not [11]. An objective antitumor effect in certain dogs with the stage IV melanoma could be also achieved by their vaccinating DNA encoding human tyrosinase [12,13]. This effect was immediately related to occurrence of canine tyrosinase-binding antibodies in the sera [14]. No autoimmune complications and serious side effects of the xenovaccinotherapy were noted in the dogs [12,13]. Noteworthy is also that this therapy could be effectively used in combination with surgical treatment [15].

The ability of xenovaccination to break the immunological tolerance to TAA has been demonstrated in a murine model of breast cancer [16]. Protooncogene HER-2/neu is a self-antigen expressed by tumors and nonmalignant epithelial tissues. DNA vaccination of mice with human HER-2/neu was found to overcome the immunological tolerance to that protein and to induce the antibody-mediated, immune responses directed against both cancer and normal breast cells [16]. Yet, vaccinations of mice with a fragment of human HER-2/neu (435-443) induced generation of the CTL able to effectively recognize the syngeneic, HER-2/neu-positive tumor cells. Importantly, the CTL generation in these experiments depended on the age of vaccinated mice [17].

Survivin, a member of the inhibitors of apoptosis, is considered as an ideal vaccinal TAA, due to its broad expression pattern in many types of human malignancies. Dendritic vaccination of mice with human survivin was shown to induce the T-helper 1-mediated, immune responses, which were markedly enhanced by depleting CD25 +, Foxp3+, CD4 + regulatory T cells. Noteworthy is that the generation of survinin-specific CTLs lacked in this model [18]. The antitumor effect of administrating human survinin in mice was also reported in the models of lymphoma [19], glioma [20, 21], and pancreatic cancer [19].

A high expression of epidermal growth factor receptor (EGFr) is attributable to different tumors including lung carcinoma and breast cancer [22]. It is likely that EGFr may be involved in autocrine and paracrine stimulation of tumor cell growth. Vaccination of mice with DNA encoding a extracellular part of EGFr was found to break the tolerance to murine EGFr and to induce immune responses directed against EGFr –positive tumor cells. The antitumor effect observed in this model was mediated both by IgG antibodies and by CTL and associated with elevation of the serum concentration of IFN-γ , as well as of IL-4 [22].

Prostate-specific membrane antigen (PSMA) is a prototypical differentiation antigen expressed on normal and neoplastic prostate epithelial cells, and on the neovasculature of many solid tumors]. Immunizations of mice with human recombinant PSMA or DNA encoding PSMA were shown to induce the production of antibodies able to bind to murine PSMA; an effective vaccination was also demonstrated with administrating the xenoantigens prepared from the tumor prostate [23, 24].

A high expression of mesothelin is attributed to pancreas cancer. Therapeutic vaccination of mice with human mesothelin was found to result in inhibition of pancreatic cancer disseminating. Such a antitumor effect was associated with the rise in serum antimesothelin antibodies, as well as with an increase in the functionality of mesothelin-specific CTL [25].

Glioma membrane proteins (HGP) are typically expressed in the cells of malignant glioma. Therapeutic vaccination of rats with human HGP was demonstrated to result in the glioma growth inhibition that was mediated by the HGP-specific Th1 cells and characterized by a pronounced infiltration of tumor tissues with CD4+ and CD8+ T cells. In contrast to the human HGP, its murine analog lacked any antitumor activity [26].

Alfa-fetoprotein (AFP) is highly expressed in liver cancer. Vaccination with the recombinant rat, but not mouse AFP was found to provide a significant prolongation of survival in

hepatocarcinoma -bearing mice. The antitumor effect of such vaccination depended on functional activity of both CD4+ and CD8+ T-lymphocytes [27].

Unlike other cancers, the neuroendocrine tumors, such as a gastrinoma, insulinoma, and medullar thyroid carcinoma, do not demonstrate any clear association with expression of defined classes of membrane-bound TAA. Therefore, for generating antitumor immune responses in such cases, the approach has been offered based on breaking the immune tolerance to tumor-derived, soluble products. In a model of thyroid carcinoma it was demonstrated that therapeutic vaccination of mice with the human (but not murine) calcitonin resulted in a significant inhibition of tumor growth. The antitumor affect of this vaccination was associated with infiltrating the tumor by calcitonin-specific CTLs, as well as with a sharp decline in the serum level of calcitonin [28].

Tumor development requires neoangiogenesis. Therefore, the therapeutic approaches aimed at preventing growth of tumor-feeding vessels are in the focus of experimental and clinical studies. Theoretically, breaking the immunological tolerance to molecules involved in angiogenesis could restrain tumor growth. A fibroblast growth factor receptor-1 (FGFr-1) is one of such molecules. Vaccination of mice with recombinant chicken FGFr-1 was reported to be able to overcome the tolerance to endogenic FGFr-1, eliciting production of FGFr-1-specific, IgG autoantibodies [29].

Matrix metalloproteinase-2 (MMP-2) is well known to play an important role in angiogenesis and to promote tumor cell expansion in the body. Immunizations of mice with the LLC or CT26 tumor cells expressing chicken MMP-2 was found found to induce antiangiogenic immune responses, resulting in the appearance of MMP-2- binding autoantibodies [30, 31].

Endoglin is a marker of angiogenesis in solid malignancies, including liver cancer. Therapeutic vaccination of mice with an extracellular portion of porcine endoglin was shown to induce immune responses directed against colorectal and lung cancers. The generation of such responses depended on functional activity of CD4+ T-lymphocytes and resulted in the appearance of endoglin-binding autoantibodies [32, 33]. A significant enhancement of antitumor effect was achieved when protein vaccination was combined with DNA vaccination. Such a combined vaccination induced not only the synthesis of endoglin-binding autoantibodies, but also the generation of endoglin-specific CTL [34]. An antitumor effect of DNA vaccination with porcine endoglin was also demonstrated in a murine model of liver cancer. This effect was mediated by both cellular and humoral immune reactions [35].

Tie-2 is an endothelium-specific receptor tyrosine kinase known to play a key role in tumor angiogenesis. Therapeutic vaccination of mice with human Tie-2 was found to be capable of exerting a negative effect on the growth of melanoma and hepatic cancer. This effect was dependent on functional activity of CD4+ T-lymphocytes and mediated by antibodies binding murine Tie-2 [36].

A pronounced antiangiogenic effect can be induced by vaccination with xenogenic whole endothelial cells [37]. This effect may be associated with overall tumor growth inhibition [38].

An antiangiogenic effect can be also achieved by inactivating soluble angiogenic molecules. For example, the vaccination of 9 dogs with spontaneous sarcomas by human endothelial cell growth factor (VEGF) resulted in the production of autoantibodies capable of binding both human and canine VEGF. The antitumor effect was observed in 3 (30%) vaccine-treated dogs. No complications of the vaccinotherapy were noted [39] .

It appears that antiangiogenic immunotherapy can be effectively combined with breaking the tolerance to differentiation antigens in order to induce clinically relevant antitumor responses. For example, the administration of DNA encoding tumor endothelial marker 8 (TEM8) was able to enhance the tumor immunity in melanoma mice, induced either by rat neu or by human tyrosinase-related protein 1 (TYRP1/hgp75) [40]

3. Clinical application of xenovaccinotherapy

Prostate cancer, melanoma, colorectal cancer and renal cancer are usually resistant to the standard cytotoxic therapy, including highly toxic combinations. On the other hand, all of these cancers express TAA which are capable of inducing antitumor immune responses. Hence, immunotherapy has to become the mainstay in treating those cancers.

Prostatic acid phosphatase (PAP) is a differentiation antigen expressed by normal and malignant cells of prostate. Patients (n=21) with metastatic prostate cancer were multiply vaccinated with autological dendritic cells loaded with mouse PAP. Such vaccinations were found to be safe to use, with no serious side effects being observed. An increased T-cell reactivity to murine PAP was observed in all of the vaccine-treated patients. Only 8 of 21 evaluable patients exhibited enhanced immune responses to human PAP. These responses associated with the enhanced production of IFN-γ and tumor necrosis factor (TNF)-α, but not IL-4 [41]

Immunologic effects of DNA vaccination with mouse tyrosinase have been assessed in 18 patients with melanoma, and the generation of tyrosinase-specific CD8+ memory T cells was found in 7 of them No serious complications of such a treatment were noted [42]

It should be noted that immunizations with one or several tumor-associated, antigenic peptides frequently fail to control overall tumor development, creating favorable conditions for growth of the tumor cell clones lacking vaccinal determinants. Moreover, due to a high lability of cancer genome, there is an antigenic diversity even in tumor cells of the same origin [43]. Since whole tumor cells express a variety of TAA and are able to elicit a broad spectrum of immune responses, they could be more applicable to constructing cancer vaccines, compared to a single or just few antigenic peptides. However, immunizations with unmodified homologous (autological or allogeneic) tumor cells have demonstrated only limited therapeutic success in cancer patients. There are two major reasons for the low immunogenicity of homologous cell vaccines. Firstly, most of the homological TAA represents self-antigens, which are not inherently immunogenic. Secondly, antigen-presenting cells do not recognize the homologous tumor cells as potentially pathogenic targets that should be internalized and their antigens processed [43].

From the aforesaid, we favor a xenogenic cell-based vaccine . Because of their structural distinctions from homologous analogs, the xenoantigens are capable of effectively overcoming the immune tolerance to self-antigens, including TAA. Yet, all humans possess natural (preexisting) antibodies, which provide an acute rejection of any non-primate cells and function as a major barrier for the transplantation of animal organs to humans [44]. A significant part of these antibodies represents the Ig G specific to the a-gal epitope that is expressed abundantly on glycoproteins and glycolipids of non-primate mammals and New Word monkeys [45]. In our point of view, by the opsonization of xenogenic tumor cells, the natural antibodies could promote internalization of tumor material in antigen-presenting cells via a Fc-receptor-mediated mechanism, and thereby enhance greatly the immunogenic presentation of TAA to tumor-specific T lymphocytes. This proposition is consistent with the published data indicating a critical role of the FcR-receptors in generating an effective

tumor immunity [46], as well as with the findings showing that the rejection of alpha-Gal positive tumor cells can efficiently boost the immune response to TAA present in alpha-Gal negative tumor cells [47].

A xenogenic polyantigenic vaccine (XPV) under study is composed of disrupted murine B16 melanoma and LLC carcinoma cells. The XPV is stored in the form of frozen-dried preparation and is suspended in physiological salt solution immediately before its administration. Since the XPV includes a wide spectrum of melanoma- and carcinoma-associated antigens, our opinion is that it may be applicable for treating different cancers.

The study with xenovaccination was performed in exact accordance with the protocol approved by the Scientific Council and Ethics Committee at the Institute of Clinical Immunology (Novosibirsk, Russia). Informed consent was obtained from every subject who underwent xenovaccinotherapy. Eligibility criteria included histologically proven, measurable disease, no prior immunosuppressive therapy for a minimum of 4 weeks, a good performance status (Karnofsky scale, 70% or more) and adequate marrow, renal and hepatic functions.

An inducing vaccinal course consisted of 10 subcutaneous immunizations (5 at weekly and 5 at fortnight intervals) and took about 3 months. Each vaccinal dose contained 75×10^6 (B16 + LLC) dead cells. Twenty-four hours following each of the first 5 vaccinations, a part of the patients was given subcutaneously a low dose of a non-oxidated recombinant IL-2. Since, when combined with XPV, IL-2 had no any significant effect on XPV-induced long-lasting immunoreactivity, its administration in the above- indicated way was recognized unpractical. Following an inducing course of the treatment each of the patients received supporting vaccinations monthly or less frequently. Throughout follow-up time the trial patients received no any systemic therapy other than immunotherapy.

A total of 152 patients with advanced forms of melanoma, colorectal or renal cancers have completed an inducing vaccinotherapy consisting of 10 immunizations and had adequate follow-up to monitor toxicity, immune responses and survival. No III-IV grade systemic toxicity associated with the vaccine administration was noted. During 24-to-48 h post vaccination only nearly 10% patients exhibited an influenza-like syndrome in the form of a small body temperature rise and musculoskeletal discomfort, which were usually self-limiting. Irritations at the injection sites were developed in the most patients in response to vaccination. Local manifestations usually disappeared within 72 h following vaccine injection. There were no treatment- related hospitalizations or mortalities.

Cell and biochemical blood parameters, as well as renal and hepatic functions, remained within the initial ranges throughout the inducing vaccinotherapy. Also there were no significant changes in subpopulation composition of PMBCs tested by immunofluorescence for expression of CD3, CD4, CD8, CD 20, and CD16 surface markers.

The development of systemic autoimmune disorders could not be excluded initially in XPV-treated patients because of the broad range of different antigens present in XPV. However, XPV-treated patients exhibited no evidence of any systemic autoimmune disorders. Their serum concentrations of a rheumatoid factor, but also of antibodies specific to DNA, cardiolipin, thyroglobulin, microsomal fraction of thyrocytes remained in the initial ranges throughout the inducing vaccinotherapy.

An inducing vaccinotherapy was found to increase detectably the serum concentrations both of IFN-γ and of IL-4. Yet it should be noted that an increase in the IFN-γ level was more common and greatly pronounced in the XPV-treated patients, compared with that in IL-4 level [48,49].

With inducing vaccinations, a remarkable increase in both T cell- and antibody-mediated immunoreactivity to vaccinal antigens was found in the majority of assessable patients. An

important aim of our study was to determine whether or not murine TAA would be capable of contributing to the generation of immune responses specific to human TAA. Indeed, our data clearly indicated that inducing vaccinations were able to significantly enhance T cell-mediated reactivity to human melanoma-associated antigens, while not affecting that to the control alloantigens [50, 51]

In our study an overall survival in the XPV-treated patients (n=51) with metastatic melanoma was evaluated through 7 year follow-up period. The control group was composed retrospectively of the patients who received conventional therapy, and had the initial clinical characteristics similar to those of the trial group. The characteristics of the trial and control patients are presented in Table 1. If it was reasonable and possible, both trial and control patients underwent a cytoreductive palliative surgery. As shown in Figure 1, the median survival in the XPV-treated patients was significantly longer than that in the control patients (14 *vs.* 6 months, respectively; $p < 0.05$). Of note is that almost all of those trial patients, who have survived for 2 years after immunotherapy initiation, further survived as long as 7 years and longer. The overall 7-year survival rate in XPV-treated and control patients was 20% and 0%, respectively. More impressing results were obtained when a long-term overall survival has been analyzed in the stage III melanoma patients (n=48; 26 females and 22 males aged from 22 to 76 years). The control group was composed retrospectively of the clinically comparable patients (n=27; 12 females and 15 males aged from 35 to 77 years). Initially, each of assessable patients had a high risk of disease recurrence. As shown in Figure 2, an overall 6 year survival rate in the trial group was 54%, whereas that in the control group only 25%. It is important to note that a better survival in melanoma patients was associated with their increased DTH to the vaccinal B16 melanoma antigens [49,50].

Characteristic	Trial	Control
Number of patients	51	32
Males/females	25/26 (49%/51%)	10/22 (31%/69%)
Age, years (median, range)	51.8±2.4 (18-72)	48,2±2,3 (24-77)
Site of metastases:		
Lung	16 (31%)	6 (19%)
Liver	10 (20%)	7 (22%)
Lymph node, skin/soft tissue	34 (67%)	26 (81%)
Other organs	8 (15%)	8 (25%)

Table 1. Characteristics of stage IV melanoma patients assessable for survival.

We also evaluated a long-term overall survival in the 35 XPV-treated patients with stage IV colorectal cancer. The control group was composed retrospectively of the patients (n=35) who received conventional therapy. Since the trial patients were very heterogenous in their clinical characteristics, each control patient was randomly selected to be a clinically comparable counterpart of a trial patient, thus control and trial groups were evenly balanced by both prognostic and clinical parameters. The characteristics of colorectal cancer patients are presented in Table 2. As shown in Figure 3, the median survival in the XPV-treated patients was significantly longer when compared with that in the control patients (18 *vs* 8 months, respectively; $p < 0.05$). An overall 2-year survival rate in the trial and control group was 27% (10 patients) and 3% (1 patient), respectively. However, patients in the trial group almost completely lost their survival advantages as early as at 3.5 years after the immunotherapy initiation.

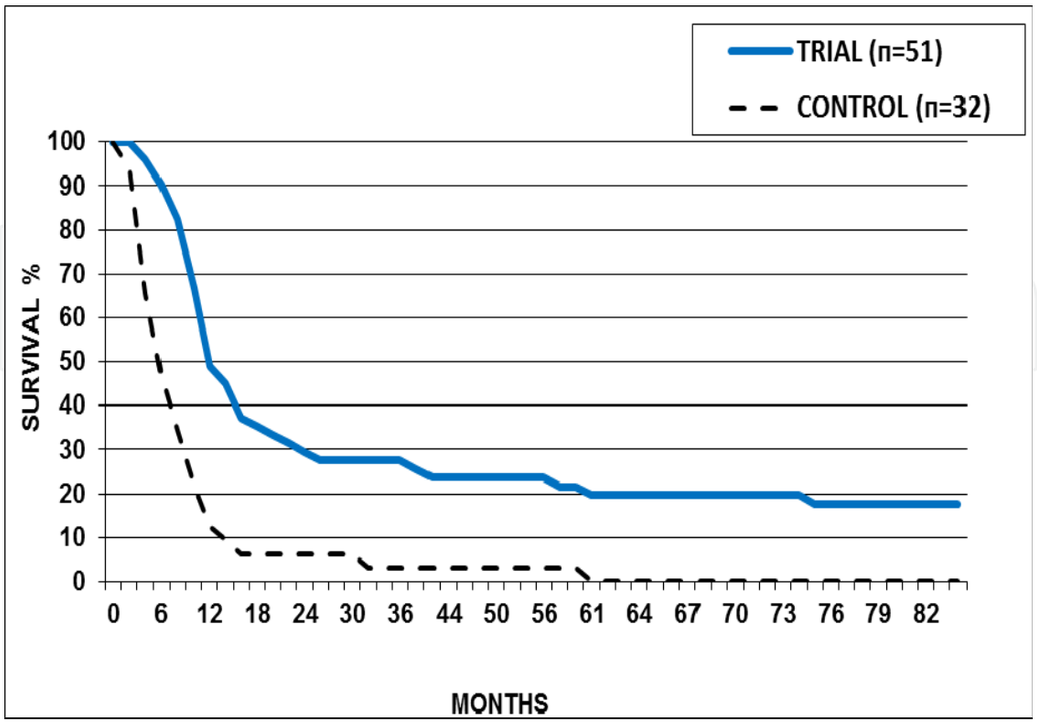

Fig. 1. Survival in the patients with stage IV melanoma. See text for further details.

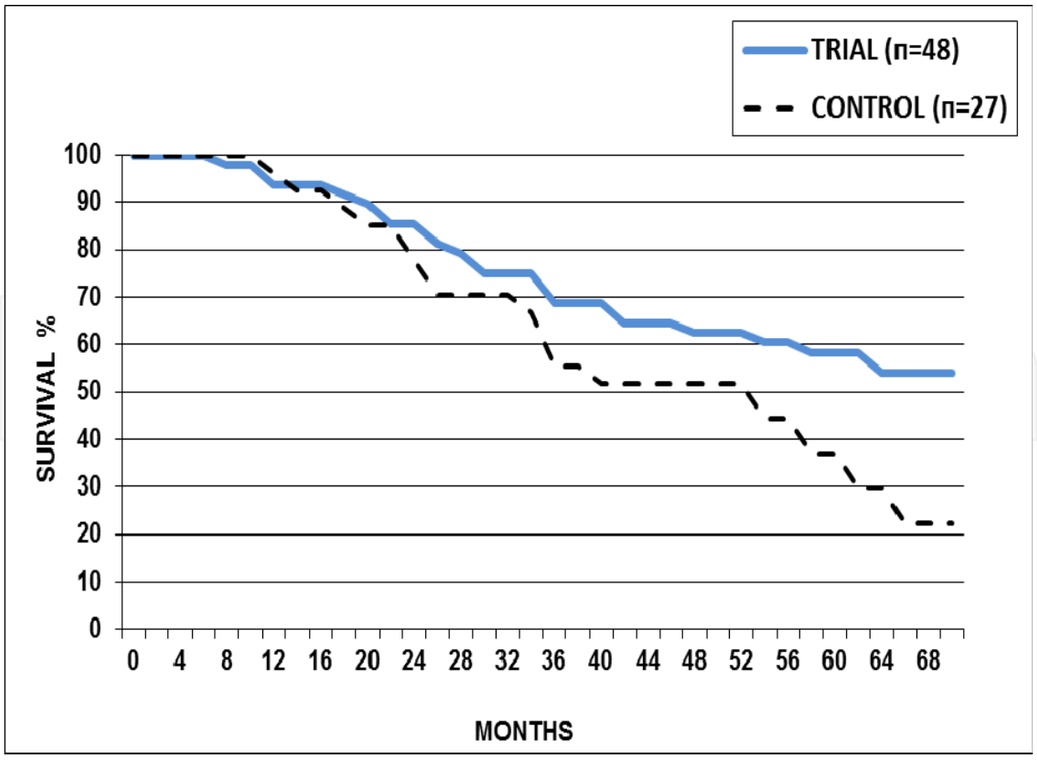

Fig. 2. Survival in the patients with stage III melanoma. See text for further details.

Characteristic	Trial	Control
Number of patients Males/females	35 19/16 (54%/46%)	35 19/16 (54%/46%)
Age, years (median, range)	61.1 ± 1.4 (38- 79)	55.6 ± 1.7 (30 - 80)
Site of metastases: Lung Liver Lymph node,skin/soft tissue Other organs	 7 (20%) 25 (71%) 17 (48%) 11 (31%)	 6 (17%) 19 (54%) 15 (43%) 8 (23%)

Table 2. Characteristics of stage IV colorectal cancer patients assessable for survival

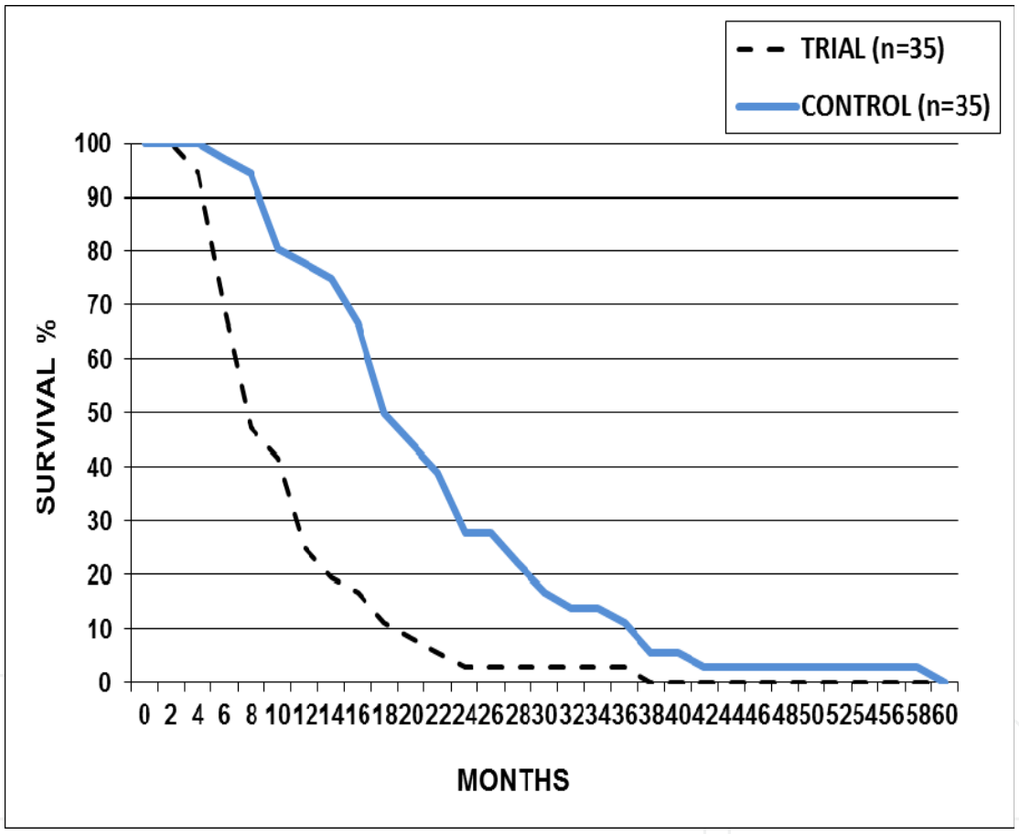

Fig. 3. Survival in the patients with stage IV colorectal cancer. See text for further details.

Figure 4 characterizes a long-term overall survival in the 16 XPV-treated patients (5 females and 11 males aged from 54 to 76 years) with stage IV renal cancer. The control group was composed retrospectively of clinically comparable patients (5 females and 11 males aged from 49 to 77 years) . The median survival in the trial patients was found significantly longer when compared with that in the control patients (20 *vs* 8 months, respectively; $p<0.05$). Noteworthy is that patients in the trial group maintained the certain survival benefits from the immunotherapy throughout 5 year follow-up period.

Overall, our results point out that the XPV-based therapy is safe for clinical use, and has no toxicity that is attributable to current standard treatments for cancer. It is also important that

the XPV-treated patients exhibited no evidence of systemic autoimmune disorders, of which a risk of development could significantly limits clinical application of polyantigenic xenovaccination .

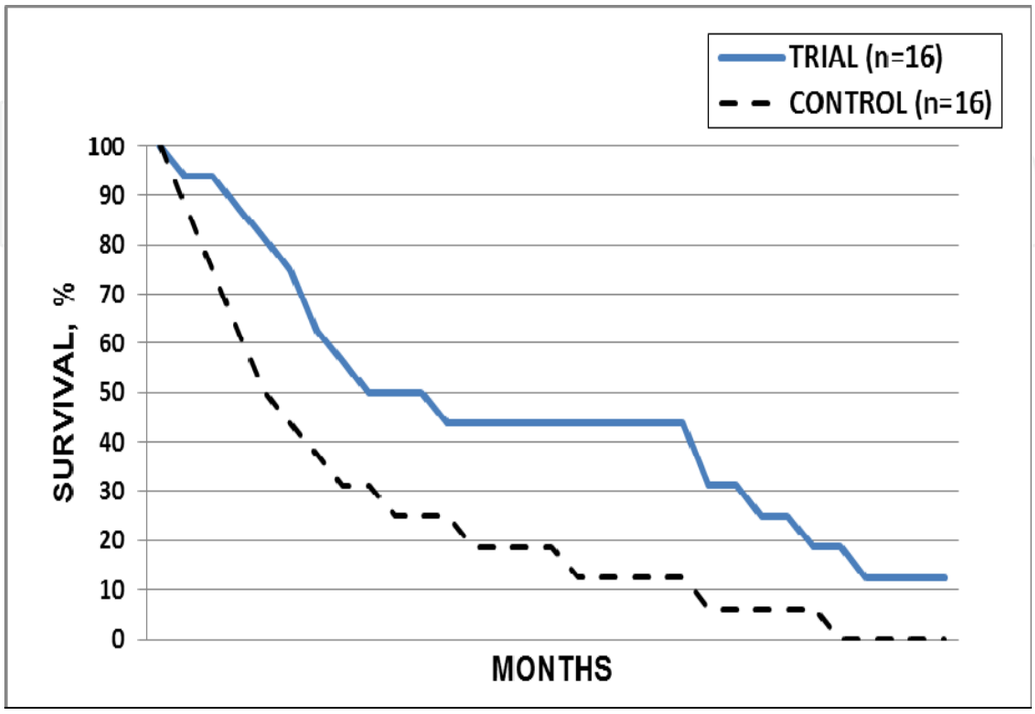

Fig. 4. Survival in the patients with stage IV renal cancer. See text for further details.

It appears that the xenogenic antigens, not only tumor-associated, but also those inherent to normal cells, can be involved in XPV-induced immune responses. As evidenced by both cell- and antibody-mediated reactions [50,51], the immune-mediated sensitization to murine TAA observed in the XPV-treated patients was detectably greater than that to murine spleen cells antigens. This may imply that the antigens associated with tumor cell phenotype might be more significant in generating XPV-driven immune processes than those being only expressed in fully differentiated cells.

Our data demonstrated that the xenovaccinations resulted in serum level elevations of not only of IFN-γ, but also of IL-4, suggesting intensification of both T helper 1- and T helper 2-mediated immune responses in XPV-treated patients [48, 49]. These findings are of great importance in the light of previously reported data that indicate a critical role for cooperating T cell- and antibody-mediated mechanisms in generating tumor cytotoxicity in vivo [46].

According to our experience [50, 51], the xenovaccinotherapy can result in generating complete or partial clinical responses in a certain portion of cancer patients. Nevertheless, stabilization of the disease appears to be the most common outcome of effective immunotherapy in advanced cancer patients. The XPV-based therapy is not an exception in this regard. Unlike the cytotoxic chemotherapy, tumor vaccine-based approaches may permit the host to reach a state of balance with the tumor, in which the net result of tumor growth and destruction is zero. That might lead to more significant survival benefits than a rapid destruction and rapid regrowth of the tumor following cytotoxic therapy.

Actually, our results suggest that the polyantigenic xenovaccinotherapy can significantly affect survival in cancer patients. It should be noted that the majority of patients entered into

our investigations were with very advanced (stage IV) disease. It is reasonable to anticipate that, as with other immunotherapies, the XPV-based therapy might be maximally effective when being applied as early as possible following surgical resection of the prime tumor. Consistent with this assertion, the most survival benefits from immunotherapy were noted in the group of stage III melanoma patients when xenovaccinotherapy was initiated before appearing distant metastasis lesions.

4. Conclusion

From the data mentioned above it appears that there are two main ways of using cancer xenovaccinotherapy: the first approach is directed on activation of the immune system against membrane-bound and soluble TAA, and the second one is aimed at overcoming the immune tolerance to the proteins that promote tumor progression. The most antitumor effects are likely to be expected when vaccinal xenogenic TAA elicitit both cellular and humoral immune reactions. The present paper is the first demonstration of the positive effect of polyantigenic xenovaccinotherapy on a long–term survival patients with advanced cancers. Although the results are extremely encouraging, they must be interpreted with caution because they are based on a small number of patients with very advanced disease.

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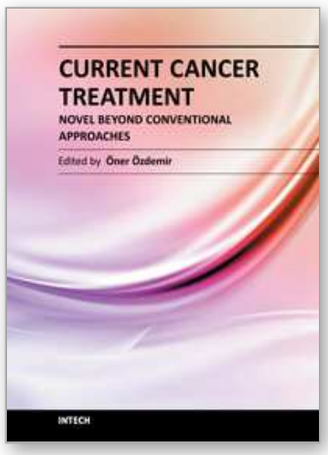

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Currently there have been many armamentaria to be used in cancer treatment. This indeed indicates that the final treatment has not yet been found. It seems this will take a long period of time to achieve. Thus, cancer treatment in general still seems to need new and more effective approaches. The book "Current Cancer Treatment - Novel Beyond Conventional Approaches", consisting of 33 chapters, will help get us physicians as well as patients enlightened with new research and developments in this area. This book is a valuable contribution to this area mentioning various modalities in cancer treatment such as some rare classic treatment approaches: treatment of metastatic liver disease of colorectal origin, radiation treatment of skull and spine chordoma, changing the face of adjuvant therapy for early breast cancer; new therapeutic approaches of old techniques: laser-driven radiation therapy, laser photo-chemotherapy, new approaches targeting androgen receptor and many more emerging techniques.

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