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Research article

Twenty-Five Years of Cancer Immunogene Therapy: Anti - Gene IGF-I Approach

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Abstract

IGF-I is one of the most important growth factors that is related to normal differentiation, and its overproduction in mature tissues is a sign of neoplastic processes, e.g. liver or brain malignant tumors. For this reason, IGF-I became an essential target for gene testing and therapeutic purpose. The development of a new medical domain in 1992/1993, called cancer gene therapy, followed by NIH and FDA approval in 1994, represents an important revolution in the treatment of malignant tumors. The new strategy, using anti-gene (antisense/triple helix) anti IGF-I technology, has shown promising results in clinical trials of malignant tumors derivatived of all three embryonic layers; in the case of tumor derivatived of neuroectodermal tissue – glioblastoma, the median survival of patients reached 21 months and, in some cases, up to three and four years. This strategy was also proven to be efficient in the treatment of mesodermal and endodermal tissue derivatives - cancers of colon, prostate and ovary.

Key words: cancer gene therapy, IGF-I, antisense, triple helix, glioblastoma, CD 8

Introduction

In the early 1970s, studies on normal or tumoral development of the brain, as well as the treatment of brain tumors, were insignificant. During the 1970s and the 1980s, researchers tackled this new challenge through a comparative study of the development of the normal and neoplastic central nervous system using a new marker, the alpha fetoprotein [1], and found a convergence between embryonic fetal development and neoplastic development [1,2]. Since the 1990s, a new oncoprotein marker, growth factor IGF-I, was localized in normal and neoplastic glial cells, and absent in neuronal cells [3,4]. IGF-I is considered

currently as the most important growth factor [5].

When it came to central nervous system tumor treatment, different strategies were applied including the use of antibodies targeting oncoproteins. This approach resulting inefficient, another approach was stopping the synthesis of oncoproteins in cancer cells directly at the "source"- i.e. translation or transcription levels. Targeting translation level was carried out through antisense technology [6-8] in the rat glioma model, using a vector that expresses IGF –I RNA antisense [4,9]. This approach has yielded positive results in vitro, stopping the synthesis of IGF-I in cell cultures, and in vivo, stopping the development of rat glioma tumor [9]. These results have given birth to a new domain of cancerology - cancer gene therapy, also referred to as cancer immunogene therapy [10]. The results obtained using antisense technique were confirmed by applying the triple helix technique targeting IGF-I on transcription level [11,12] and using the same model of rat glioma [13]. These results lead to the approval for clinical trial by the NIH and FDA, initially for glioblastoma treatment [9,10].

Immunotherapy and immunogene therapy of cancer are the latest approaches for the treatment of various forms of malignant tumors. Since 2015, immunotherapy has become one of the most relevant cancer treatments in the U.S. through the launch of programs like "Cancer Moonshot". The start of our phase I trial of malignant brain and liver tumors using IGF-I antisense / triple helix strategy (U.S.A, Europe and China) has shown promising results. Indeed, an increase in immune response against tumors was demonstrated, accompanied by a remarkable increase in patient survival [14,15].

Theoretical framework

"The true journey of discovery does not consist in seeking new landscapes, but in having new eyes" (A la recherche du temps perdue, Marcel Proust, 1918).

Receptors and signaling pathways of growth factors are frequently overexpressed in various neoplasms and can contribute to oncogenesis. Growth Factor IGF-I plays a key role in neoplastic growth. IGF-I is a polypeptide of 76 amino acids, which participates in the differentiation of cell and tissue [3,5]; it is considered that 17 different tumors express the IGF-I gene (i.e. brain tumors such as glioblastoma, and colon, prostate and ovary cancer) [9].

Our strategy towards the study of neoplastic development has focused on the Anti – Gene technology (anti IGF-I gene), either of antisense type (AS) or of triple helix type (TH), both of which stop the synthesis of IGF-I in transfected cancer cells [4,6,11]. The transfected AS and TH cells, when re-implanted in vivo, have induced a specific anti-tumor immune response. The mentioned results have been observed as well in animal model as in clinical trials of malignant tumors [9,14,16,17].

The results obtained so far with classical treatment of malignant tumors, especially of glioblastoma (surgery and chemotherapy) are unsatisfactory, as the survival of these recurrent patients is discouraging low. In order to find a specific solution for cancer treatment, the purpose of our strategy was to evaluate the implementation of immunogenic therapy, concomitant with radio and chemotherapy. The objective has been to improve quality of life, based on the hypothesis of increased survival, due to the possible increase in tumor control, and the absence of neurological and/or general symptoms derived from both the toxicity of surgery and chemotherapy [14].

New therapies of malignant tumors include the use of inhibitors (imatinib, gefitinib) including antibodies (i.e. Avastin), antisense oligonucleotides, peptides and other molecules, and especially cell immunotherapy targeting growth factors and their receptors [16-26]. However, although the average survival of glioma treatment has reached almost a year and a half, we are still far from a cure [17, 27-34].

As to the new strategies towards the treatment of malignant tumors, the approach of anti-gene therapy is related with targeting the genes of growth factors expressed in these tumors, specifically IGF-I, TGF Beta, VEGF or EGF [16,26,27]. Suppressing the expression of IGF-I in tumors leads to an antitumor immune response superior to that of the other growth factors [5,16,35].

Our article focuses on the methodology of Anti IGF-I gene therapy, analyzing our different and previous basic and clinical results, obtained in Europe, USA, and Asia [NATO Science Program – Gene Therapy, USA, France, Poland, Germany, not LST 980517]. A better understanding of the "anti-gene strategy against IGF-I" has allowed us to achieve one goal: increasing the current survival of patients with glioblastoma and establish the standardization of the anti-IGF strategy-I in the treatment of cancer in clinics in Europe and Colombia. This established common criteria for the selection of vaccines was the expression of MHC-I, including TAP-1 and -2, B7. Moreover, other criteria were the blood cell markers PBL in treated cancer patients:

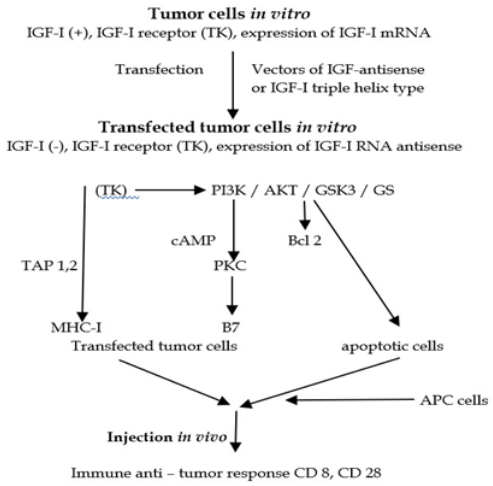

Figure 1. Schema of cancer immunogene therapy using IGF-I antisense / triple helix approach. Culture of cancer cells, expressing IGF-I and IGF-I-Receptor, is transfected with vectors of 'antisense' or 'triple helix' type. The transfected cells, negative for IGF-I, over-express IGF-I-R (Tyrosine Kinase) inducing signal transduction pathway. The transfected cells become immunogenic, expressing MHC-I and B7, and partially apoptotic. Both populations, immunogenic cells and apoptotic cells, while injected in vivo, induce immune anti-tumor response

Methodology

Anti - gene technology

An efficient strategy was established by construction of vectors targeting growth factors present in neoplastic develop-

ment. Anti-Gene technology was applied to construct the vectors expressing either IGF-I antisense RNA or IGF-I RNA forming RNA-DNA triple helix [4,13]. The vectors introduced in the cells in vitro, enable to completely stop the synthesis of growth factor on translation or transcription level, respectively. Using this methodology, autologous tumor cells modified ex vivo by mentioned vectors expressed in vitro MHC-I and B7 antigens; these cells, after three weeks of in vitro culture, have begun to show apoptotic symptoms. When injected in vivo in animals bearing tumors or applied in clinical treatment of glioblastoma patients, these genetically modified cells induced an immune anti-tumor effect (CD8+) [Figure 1]. Such immune response has produced the arrest of tumor development in animals and an increase of the median survival of patients [33] (successful clinical results obtained in EE.UU., E.U., China).

The principal strategies of cancer gene therapy for treatment of malignant tumors especially gliomas, including antisense approach, have been proposed since the 1990s – 2000s [4,36-39]. Although different antisense approaches have often given satisfactory results in animal models, only IGF-I antisense and triple helix have demonstrated clinical oncology results [17].

Antisense approach

Antisense approach was "discovered" by the groups of F. Jacob and R.M. Harland [6,7]. It has been proven that a lot of genes present an open reading frame on the antisense strand [40,41], and a natural antisense RNA in prokaryotes could play a regulatory role in replication, transcription or translation steps of some genes [7].

An antisense RNA, hybridized on its complementary sequence in a mRNA blocks the ribosome progression during the translation of the mRNA. This observation constitutes the "starting point" of the antisense or non-sense approach [6] based on antisense RNA or antisense oligonucleotides to modulate artificially and specifically the expression of genes. The plasmid vector allows the intracellular transcription of antisense RNA which can strongly hybridize to the mRNA and stop the translation. Generally, an effective inhibition demands a high copy number of antisense RNA relative to mRNA. In our antisense anti IGF-I approach, we have efficiently stopped IGF-I mRNA, and the same the synthesis of IGF-I protein, because the used AS vector has produced more than 50 copies of antisense RNA by cell [4,42]. The chemical stability of plasmid-derived antisense RNA seems much more efficient than that of antisense oligonucleotides delivered directly into cells. The antisense oligonucleotides are exposed to intra- and extracellular nuclease activity [43].

The first antisense oligonucleotide used in clinical pharmacology was as anti-cytomegalovirus therapy (VitraveneTM) (Vitravene Study Group, 2002). The examples of antisense therapy, especially useful in research and clinical studies concerning human malignancies, were largely described [44]. As mentioned previously, when it comes to antisense anti-tumor therapy, different strategies have been applied since from 1990-2000s. Among them were strategies based on: antisense of genes encoding growth factors i.e. IGF-I, TGFbeta, EGF, VEGF [9,26,45-47]; antisense oncogenes i.e. c-myb, bcr/abl, ras [43,48-50]; antisense of apoptosis related elements i.e.bcl-2 [51,52]; antisense of protein as for example PTHrP, or proteins related to MHC expression [53, 54]; and antisense of genes encoding enzymes [55].

Triple helix approach

The triple helix (TH) technology belongs together with the antisense approach to anti-gene strategies. The TH technology was "discovered" by groups of P.B. Derwan and of C. Helene [11,12]. Its action is defined by gene inhibition at the translation level as follows: the short specific oligonucleotides (so called triple-helix forming oligonucleotides, TFOs) are delivered to cells both by cell transfection with chemical carriers and via vector plasmid that can drive the synthesis of TFO RNA. TFOs link to genomic double-strand DNA, form triple-helix structure with target gene and strongly inhibit its expression at transcriptional level. A triple-helical structure on DNA is considered to block transit of RNA polymerase. TFOs are usually targeted against polypurine/polypyrimidine sequences located in control regions (promoters) of the genes of interest [11,56,57] .

Since the 1990s, the triple helix strategy has been applied to different targets as follows: ras, HERE-2/neu/ oncogenes [58-61]; and growth factors i.e. IGF-I, TNFalpha, EGFreceptor [13,14,62,63]. In 1990s - 2010s triple helix strategy has been successfully introduced in experimental and clinical gene therapy trials [63-65].

A novel development in oligonucleotide technology is the finding that 21–23-mer double-stranded RNA molecules, known as siRNA, can effectively silence gene expression [66]. The role of 22-23 mer RNA in the silencing of gene is strongly similar to that of triple helix RNA-DNA mechanism involving also 23 mer RNA [12,67].

Presentation of anti - gene IGF-I clinical protocol

Our clinical protocol has concerned malignant tumors of all three embryonic derivatived tissues, neuroectodermal, mesodermal and endodermal. In the Phase I, three to five cases of cancer diseases have been treated: glioblastoma, cancers of prostate, ovary and colon.

Malignant tumors

Glioblastoma

The incidence of this malignant brain tumors in the world is 3-8 cases per 100,000 people. It is the third leading cause of cancer mortality. The primary treatment of standard glioblastoma consists of surgery as extensive as possible, followed by radiation therapy, associating chemotherapy based generally on Temozolamide. Even with aggressive combined treatment the median survival is only 12-15 months, with an average survival at five years less than 5% [17].

Prostate cancer (adenocarcinoma)

The adjusted mortality rate for prostate cancer was 12 per 1.000.000 men. Treatment may include surgery, radiation therapy, chemotherapy, or a combination of all. In chemotherapy treatment, a partial objective response is obtained between 10% and 40% of cases. Chemotherapy is not indicated as treatment for early prostate cancer [68, 69].

Ovarian cancer

Ovarian carcinoma is the most common type - 95% of

cases. Currently it was present in about 1 million women and resulted in 150 000 deaths worldwide. Among women it is the seventh-most common cancer. The typical age of diagnosis is 63. Treatment usually includes combination of surgery, radiation therapy, and chemotherapy. The overall five-year survival rate in the United States is 45% [69].

Colon cancer

Colorectal cancer (adenocarcinoma) is the third most common form of cancer and the second most important cause of mortality associated with cancer in the Americas. Colorectal cancer causes 694,000 deaths worldwide each year. Treatment is usually surgical, and in many cases is followed by chemotherapy [69,70].

Preparation of cell "vaccines"

The cell "vaccines" were prepared from cultured autologous cancer cells originated from tumor biopsies of cancer patients. The removed cancer tissue material was vial to establish the cell culture if done before 24 hours following surgery. Two-three millimeters diameter biopsies were placed in DMEM+F12 medium containing high glucose concentration. Specimen were then transferred to PBS containing collagenase, incubated for 20 min, centrifuged, and the pellet resuspended and then culturesd in 20% bovine serum in DMEM/F12. Three million cells were seeded per well in gelatin-covered 6 well plates. Using antisens /triple helix IGF-I expressing vectors (50:50), transfection was done by either Ca++/Ph technique or FuGENE 6 transfection reagent (Boehringer Mannheim). The selection of transfected cells was done in the presence of Hygromycin B (Boehringer Mannheim). The cultures of these transfected cells, serving as "vaccines", four weeks after transfection have presented about 50-60% of apoptotic cells, and 40-50% of non-apoptotic cells which were IGF-I (-) and MHC(+) [14,17].

Clinical trial

For the clinical trial protocol presented here, patients, age 17-70 years were included, and were treated by surgery followed by radiation therapy or accompanied by low doses of chemotherapy (2 months), and then by cell immunogene therapy (4 months). Total treatment for all types of cancer diseases was six months.

First of all, the selected patients present confirmed astrocytoma IV diagnostic (glioblastoma), ovarian carcinoma, prostate adenocartcinoma or colon adenocartcinoma. The selected patients have not been previously treated with corticotherapy or chemotherapy (the interference of these therapies could diminish the efficiency of immune therapy; moreover, this could impede the correct evaluation of the role of immune therapy in cancer treatment).

In the case of glioblastoma, the post-surgery treatment was composed of an obligatory radiotherapy applied according to the classical protocol for glioblastoma treatment: radiotherapy has started two - three weeks after surgery and consisted of two months of radiation. During this period of radiotherapy the patients were treated also with chemotherapy using a low dose of temozolomide. In the case of colon, prostate and ovary cancers, the patients have followed classical protocols. No radiotherapy and no chemotherapy was applied after surgery. Two months after surgery, the cell immunogene therapy was introduced [71].

The immunotherapy was used with three subcutaneous injections into the left arm (1 ml of physiological solution containing 0,2 million transfected cells (50:50 anti - IGF-I antisense / triple helix). The injections were applied with an interval of 4 weeks; 48 hours before each vaccination, the cells were irradiated with 5000 cGy gamma (Co60 or Cs137). The blood samples were removed to mark PBL cells (peripheral blood lymphocytes) before and after each immunotherapy injection: labelling done by flow cytometry has concerned: CD8, CD8 / CD4, CD8+11b+ / CD8+11b-, CD 28, CD3, CD19, CD45 [14,33].

Results

Our strategy of treatment of malignant tumors was based on: 1) diagnosis using IGF-I gene expression as differential marker, and 2) enhancement of tumor using antisense and triple helix anti - IGF-I technology. In this type of immunogene therapy, the tumor cells were down-regulated in production of IGF-I when transfected with vectors either expressing IGF-I antisense RNA or inducing IGF-I RNA-DNA triple helix. Moreover, the transfected tumor cells become apoptotic (50 % of transfected cells). These injected cells induced a T-cell mediated immune reaction (Figure 1).

The first clinical trial concerned glioblastoma patients [63,72] followed by clinical trial of colon, prostate and ovary cancer patients [71]. In our clinical trials, the median survival of the glioblastoma patients was 21 months. The glioblastoma patients included in the control group, without immunotherapy, have survived about 10 months, coming from surgery followed by radiotherapy only. The results observed in the control group of glioblastoma patients, not treated by immunotherapy were not so different from those obtained using a classical treatment composed of surgery, radiotherapy and chemotherapy (high dose of temozolomid). In this case, using high dose of chemotherapy, median survival is generally 10 -11 months, rarely 13 months.

Admitting that group of glioblastoma treated patients, using immunotherapy, has given promising results, all colon prostate and ovary cancer patients treated with this type of immunotherapy were supervised clinically up to two years. At 20 months, all colon, prostate and ovary cancer patients treated by surgery and followed by cellular immunotherapy were alive and the treatments were well tolerated. The only secondary effect observed in the treated cancer patients, including glioblastoma patients, using cellular immunotherapy, was that of increased temperature up to 38-39 C° persisting during two-three days after every of cell vaccination. No other secondary effects were registered [17,71].

Clear-cut phenotypic changes in peripheral blood lymphocytes (PBL) was observed in all cancer patients treated with immunotherapy: after the first cell vaccination, the increased level of CD8 was registered, particularly CD8+11b-. There was a characteristic switching from CD8+11b+ to CD8+11b-. This increasing switching was also observed after the second and after the third cell vaccination in all malignant tumor treated patients (Table 1). The results concerning other studied CD molecules as CD3, CD19, CD45 (data not shown) were non-significant in all cancer treated patients; in the case of CD4 slightly decreased

values were registered.

Both IGF-I anti-gene therapies: IGF-I antisense and IGF-I triple helix, were introduced in clinical trial of glioblastoma (Cleveland, USA; Bangkok, Thailand; Bydgoszcz, Poland – collaboration with Paris, France), liver hepatocarcinoma (Shanghai, China, and Cracow, Poland), ovary, prostate and colon cancers (Bydgoszcz and Cracow, Poland), and glioblastoma, colon, prostate and epidermoid cancers (Bogota, Colombia - clinical study in progress).

Discussion

The 'creation' of gene therapy approach [73] followed by 'creation' of stem cell gene therapy [74] and finally the 'creation' of cancer gene therapy [4,9] have opened a new era of treatment for different diseases and especially cancer diseases [75-78]. Cancer gene therapy, being sensu stricto cancer immunogene therapy, was applied in clinical trial in parallel with cancer immunotherapy [29,79,80].

As far as tumors of the nervous system are considered [81-89], among the new therapy strategies based on molecular biology and chemical techniques to treat tumors of the nervous system like glioblastoma, and other malignant tumors, especially, in the case of IGF-I, TGF-beta, VEGF or EGF [5,26,77,95-97] the use of a specific approach against growth factors [23,26,33,35,98-103], their recipients [104-108], and the transduction of its signaling elements [17,24,49,50,58,93,109] seem to offer hope of a promising solution for.

The mechanism of antisense therapy targeting growth factors and their receptors (IGF-I, TGF-beta, EGF, IGF-I–R, EGF-R) constitutes a combination of increased anti-tumor immune response (CD8+) and inhibition of the transduction pathway of the PI3K/AKT/GWK3/GS signal that is involved in the transformed phenotype of the tumor [25,44,110] (Figure 1). As to CD8+ cell action, to exercise its cytotoxic effect, the formation of a bridge between this and class I antigens of the major histocompatibility complex (MHC I) is necessary [16,111,112]. For this reason, the strategy of treatment of glioblastoma with IGF-I-Receptor was not continued; it seems that this therapy could be more efficient if the "cell vaccines" were prepared after cloning of IGF-I-Receptor 'antisense' cells for expression of MHC-I.

Another aspect of our applied immunotherapy - security of clinical trial - was also related directly to used anti – gene technology. The safety of the method in cell engineering is guaranteed by the use of an episomal vector. Other techniques

Table 1. Flow cytometric 'FACS' peripheral blood lymphocyte CD marker patterns following cellular immunogene therapy in three tissue derivatived human cancers (neuroectodermal, mesodermal and endodermal): glioblastoma multiforme, prostate adenocarcinoma, ovarian carcinoma and colon adenocarcinoma. The time of established cell line as well as the time of established transfected cell line (vaccine) are mentioned in weeks. CD molecules were labelled in peripheral blood lymphocytes (PBL) obtained from pre-vaccinated patients (C – control) and after two successive vaccinations (V1 and V2) in the same cancer patients. Two cases of each of the designated cancers were examined. Flow cytometry analysis data are expressed as percent of positive cells when compared to the isotype control. Differences in percentage of CD8+ CD11b- and CD8+ CD11b- subpopulations before and after vaccinations were strongly significant.

Name of patient	Age	Type of treated cancer	Established cancer cell line	Transfected AS/TH cells	PBL cells CD8+ CD11b+	PBL cells CD8+ CD11b-
B.P.	67	glioblastoma	4 weeks	4 weeks	C-23% V1-21%, V2-20%	C-14% V1-24% V2-29%
A.W.	66	glioblastoma	4 weeks	4 weeks	C-24% V1-21%, V2-19%	C-15% V1-23% V2-27%
R.D.	65	prostate adeno-carcinoma	5 weeks	4 weeks	C-26% V1-21% V2-20%	C-12% V1-24% V2-25%
K.S.	53	prostate adeno-caecinoma	5 weeks	5 weeks	C-25% V1-22% V2-20%	C-13% V1-24% V2-27%
B.K.	57	ovarian carci-noma	4 weeks	4 weeks	C-24% V1-20% V2-19%	C-13% V1-22% V2-26%
B.H.	50	ovarian carci-noma	4 weeks	3weeks	C-26% V1-22% V2-19%	C-15% V1-24% V2-28%
A.M.	56	colon adenocar-cinoma	5 weeks	5 weeks	C-26% V1-25% V2-21%	C-10% V1-25% V2-30%
S.K.	48	colon adenocar-cinoma	4 weeks	4 weeks	C- 27% V1-25 % V2- 22%	C-11% V1-24% V2-29%

of gene therapy, are generally based on retroviral vectors that theoretically involve the risk of their integration into the DNA by the absence of episomal vectors. This important aspect of used technique was pointed out by Ethical Committee of NIH in 1993 (no 1602 (ref. 58 FR 44098) and F.D.A. (ref. BB-IND 5372), accepting anti - gene IGF-I expression vectors [9,14].

Among new investigated techniques based on molecular biology, potentially useful for clinical trials seem to be siRNA (small interfernity RNA) [113] and miRNA (microRNA) [114]. The role of these siRNAs in gene silencing is very similar to that of the TH mechanism [11,12]. Currently the use of the small transfer RNA (siRNA), has not shown definite clinical results. The siRNA can involuntarily defocate the target because it is structurally related to the microRNA. It can also result in nonspecific events due to the activation of the innate immune response. As regards miRNAs, they can play a key role in tumorigenesis, cell proliferation control and apoptosis [115]. Whether or not the miRNA technology replaces the use of oligodesoxinucleotides remains in question at this time.

Conclusion

In the presented article we have summarized the search of a solution for treatment of cancers and especially brain tumor glioblastoma, admitting that current classic therapies including chemotherapy have not given satisfactory results. At first we have comparatively studied the normal and neoplastic development of central nervous system using the new markers AFP and IGF-I [2,42,119]. Finally, IGF-I was revealed to be the main marker of neoplastic glial development, and the principal target for glioblastoma diagnostic and therapy [3,4]. We have stopped its expression on molecular levels, transcription and translation, by antisense and triple helix technology, respectively [9,13]. A new area of oncology was born – cancer gene therapy, or cancer immunogene therapy [10].

In conclusion, our article draws attention to studies in the area of cancer immunogene therapy of malignant tumors, especially glioblastoma using anti-gene approach (AS, TH,) alone or combined with pharmacological treatment [30, 116-118].

In China (2nd Military Hospital Shanghai), the survival of patients with hepatocarcinoma treated with IGF-I antisense immunotherapy was 5 years [14,15]. In the case of glioblastoma, using the proposed protocol, the average survival was 20 to 24 months, and in some cases up to 3 or 4 years, while the current treatments show the survival of 15 months [118].

The different cancer gene therapy approaches and their clinical applications are in permanent development [10].

Ethical Committees

The approval for the gene therapy clinical trial (based on NIH clinical protocol n° 1602, Bethesda, Maryland, 24/11/1993), containing scientific basis of methodology, cell therapy product standardization of preparation, detailed clinical protocol including inclusion criteria and exclusion criteria (i.e. HIV and EBV active infection) and the letter of agreement, was administrated by the Bioethical Commissions of the L. Rydygier Medical University, Bromberg (Bydgoszcz), Jagiellonian University, Cracow, Poland, no KB/176/2001, 28/06/2002, and no KBET/184/L/2000, 21/09/2000; La Sabana University, Chia, Colombia, no P 004-10, 15/12/2010; Cartagena University Hospital of the Caribbean (preclinical study), Colombia, no 3-19/10/2011; and registered by international Wiley Gene Therapy Clinical Trial database, Stockholm, no 635 and 636 (J Gene Med, updated 2002). The protocol was verified by Ministry of Health, AFSSAPS Committee, Paris, France, 03/06/2005, and by NATO Science program 2003-2007, no LST 980517. Recently the clinical protocol of anti - IGF-I cancer gene therapy was approved by Ethical Committee of CIO – Center of Oncological Investigations, Bogota, Colombia, no AVAL-009-2019, 11.04.2019

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Trojan J, Uriel J. (presented by F. Jacob). Intracellular localisation of alphafeprotein and Serum albumin in the central nervous system of the rat during fetal and post-natal development. (in French). Compte Rendu Acad Sci Paris. 1979;289(15):1157-60.
2. Trojan J, Uriel J, Deugnier MA, et al. Immunocytochemical quantitative study of alpha- fetoprotein in normal and neoplasic neural development. Dev Neurosci. 1984;6:251-9.
3. Kiess W, Lee L, Graham DE, et al. Rat C6 glial cells synthesize insulin-like growth factor I (IGF-I) and express IGF-I receptors and IGF-II/mannose 6-phosphate receptors. Endocrino. 1989;124:1727–36.
4. Trojan J, Blossey BK, Johnson T, et al. Loss of tumorogenicity of rat glioblastoma directed by episome-based antisense cDNA transcription of insulin-like growth factor I. Proc Natl Acad Sci USA. 1992; 89(11):4874-8.
5. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nature Rev Cancer. 2004;4:505–18.
6. Rubenstein JL, Nicolas JF, Jacob F. Nonsense RNA: a tool for specifically inhibiting the expression of a gene in vivo. Compte Rendu Acad Sci Paris III. 1984;299, 271-274.
7. Weintraub H, Izant, Harland R. (1985). Antisense RNA as a molecular tool for genetic analysis. Trends Genet. 1985;1(1);23-25.
8. Green PJ, Pines O, Inouye M. The Role of Antisense RNA in Gene Regulation. Ann Rev Biochem. 1986; 55:569-597.
9. Trojan J, Johnson TR, Rudin SD, et al. Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense Insulin like Growth Factor I RNA. Science. 1993;259:94-7.
10. Wikipedia - Gene Therapy, History 1990s - 2010s.
11. Dervan P. Reagents for the site specific cleavage of megabase DNA. Nature. 1992;359:87-8.
12. Hélène C. Control of oncogene expression by antisense nucleic acid.

Eur J Cancer, 1994;30A,1721

13. Shevelev A, Burfeind P, Schulze E, et al. Potential triple helix mediated inhibition of IGF I gene expression significantly reduces tumorigenicity of glioblastoma in an animal model. Cancer Gene Ther. 1997;4(2),105 12.
14. Trojan J. Anti -Gene anti IGF-I technology applied for cancer immunotherapy. World J Res Rev. 2016; 1(3):67-75.
15. Trojan LA, Ly A, Upegui-Gonzalez L, et al. Antisense anti IGF-I therapy of primary hepatic cancer. J Afr Cancer (AJC). 2008/9;1:1-10. .
16. Pan Y, Trojan J, Guo Y, et al. Rescue of MHC-1 antigen processing machinery by down-regulation in expression of IGF-I in human glioblastoma cells. PLoS ONE. 2013;8:ID:e58428.
17. Trojan J, Cloix J-F, Ardourel MY, et al. Insulin-like growth factor type 1 biology and targeting in malignant glioma. Neurosci. 2007;145 :795-811.
18. Parga C, Gaitán R, Marrugo J. Furanonaftoquinonas aisladas de Tabeuia Billbergi y evaluación de su actividad inmunomoduladora sobre células mononucleares de sangre periférica. Acta Méd Col. 2002; 27(5):311.
19. Kjaergaard J, Wang LX, Kuriyama H, et al. Active immunotherapy for advanced intracranial murine tumors by using dendritic cell-tumor cell fusion vaccines. J Neurosurg. 2005;103:156-64.
20. Rincon OL, Pareja LR, Jaramillo S, et al. Human papillomavirus, immune response and cervical cancer: a complex relationship. Rev Col Obstetric Ginecol. 2007;58(3):58-68
21. Vega EA, Graner MW, Sampson JH. Combating immunosuppression in glioma. Future Oncol. 2008; 4(3): 433-442.
22. Schlingensiepen KH, Fischer-Blass B, Schmaus S, et al. Antisense therapeutics for tumor treatment: the TGF-beta2 inhibitor AP 12009 in clinical development against malignant tumors. Rec Result Cancer Res. 2008;177:137-50.
23. Hau P, Jachimczak P, Bogdahn U. Treatment of malignant gliomas with TGF-Beta2 antisense oligonucleotides. Expert Rev Anticancer Ther. 2009;9:1663-74.
24. Arboleda G, Cárdenas Y, Rodríguez Y, et al. Differential regulation of AKT, MAPK and GSK3 during C2-ceramide-induced neuronal death. Neurotox. 2010;31(6):687-93.
25. Trojan J, Anthony DD. Antisense and neuro-oncology challenge: how to stop glioblastoma development. Curr Signal Transd Ther. 2011;6(3): 411-23.
26. Schlingensiepen KH, Jaschinski F, Lang SA, et al. Transforming growth factor-beta 2 gene silencing with trabedersen (AP 12009) in pancreatic cancer. Cancer Sci. 2011;102(6):1193-1200.
27. Baserga R. The Insulin-Like Growth Factor-I Receptor as a target for cancer therapy. Expert Opinion Ther Targets. 2005;9:753-68.
28. Cavazzana-Calvo M, Hacein-Bey-Abina S, Fischer A. Ten years of gene therapy: thoughts and perspectives. Med Sci (Paris). 2010;26:115-8.
29. Dietrich PY, Dutoit V, Tran Thang NN, et al. T-cell immunotherapy for malignant glioma: toward a combined approach. Curr Opinion Oncol. 2010;22:604-10.
30. Piwecka M, Rolle K., Wyszko E, et al. Nucleic acid-based technologies in therapy of malignant gliomas. Curr Pharm Biotech. 2011;12:1805-22.
31. Catuogno S, Esposito CL, Quintavalle C, et al. Nucleic acids in human glioma treatment: Innovative approaches and recent results. J Signal Transduct. 2012; ID: 735135.
32. Caruso G, Caffo M. Antisense oligonucleotides in the treatment of cerebral gliomas review of concerning patents. Recent Patents on CNS Drug Discovery. 2014
33. Trojan A, Jay LM, Kasprzak H, et al. Immunotherapy of malignant tumors using antisense anti-IGF-I approach: case of glioblastoma. J Cancer Ther. 2014;5:685-705.
34. Trojan J, Castillo T, Quintero G, et al. In vitro technical aspects of Anti – Gene IGF-I vaccines against glioma. Insights Biomed Res, 2019; 3(1), 43-8.
35. Moro-Sibilot DM, Coudurier M, Lantuejou S. Targeting Insulin-like Growth Factors in the treatment of cancer. Rev Malad Respir. 2010; 27: 959-63.
36. Culver K W, Rarn Z, Wallbridge S. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science. 1992; 256: 1550-2.
37. Resnicoff M, Sell C, Rubini M, et al. Rat glioblastoma cells expressing an antisens RNA to the insulin-like growth factor receptor are non tumorigenic and induce regression of wild-type tumors. Cancer Res. 1994;54: 2218-22.
38. Resnicoff M, Coppola D, Sell C, et al. Growth inhibition of human melanoma cells in nude mice by antisense strategies to the type-1 IGF-I receptor. Cancer Res. 1994;54:48-50.
39. Komata T, Kanzawa T, Kondo Y, et al. Telomerase as a therapeutic target for malignant gliomas. oncogene. 2002;21(4):656-63.
40. Merino E, Balbas P, Puente JL, et al. Antisense overlapping open reading frames in genes from bacteria to humans. Nucleic Acids Res. 1994; 22(10):19-77.
41. Yomo T, Urabe I. A frame specific syrnmetry of complementary strands of DNA suggests the existence of genes on the antisense strand. J Mol Evol. 1994;38(2):113-20
42. Trojan J, Johnson T, Rudin SD, et al. Gene therapy of murine teratocarcinoma: separate functions for insulin-like growth factors I and II in immunogenicity and differentiation. Proc Natl Acad Sci USA. 1994; 91:6088-92.
43. Galderisi U, Cascino A, Giordano A. Antisense oligonucleotides as therapeutic agents. J Cell Physiol. 1999;181:251-7.
44. Trojan J. Cancer immunogene therapy. Anti - gene anti IGF-I approach. Case of glioblastoma. Ed. Lambert Academic Publishers, Germany; 2017: pp 1-140, ISBN-13: 978-3-330-06345-7.
45. Le Roith D, Zumkeller W, Baxter RC. Insulin like-Growth Factor I. Ed. Landes Bioscience/ Eurekah & Kluwer Academic/ PlenumPublishers, New York. 2003; 1-484.
46. Wu HP, Feng GS, Liang HM, et al. Vascular endothelial growth factor antisense oligodeoxynucleotides with lipiodol in arterial embolization of liver cancer in rats. World J Gastroenterol. 2004;10(6):813-8.
47. Gu S, Liu CJ, Qiao T, et al. Inhibitory effect of antisense vascular endothelial growth factor 165 eukaryotic expression vector on proliferation of hepatocellular carcinoma cells. World J Gastroenterol. 2004; 10(4):535-9.
48. Calabretta B, Skorski T, Ratajczak M, et al. Antisense strategies in the treatment of leukemias. Sem Oncol. 1996;23(1):78-87.
49. Abaza MS, Al-Attiyah RJ, Al-Saffar AM, et al. Antisense, oligodeoxynucleotide directed against c-myb has anticancer activity and potentiates the antiproliferative effect of conventional anticancer drugs acting by different mechanisms in human colorectal cancer cells. Tumour Biol. 2003;24(5):241-57.
50. Morioka CY, Saito S, Machado MC, et al. Antisense therapy specific to mutated K-ras gene in hamster pancreatic cancer model. Can it inhibit the growth of 5-FU and MMC-resistant metastatic and remetastatic cell lines?. In Vivo. 2004;18(2):113-7.
51. Nahta R, Esteva FJ. Bcl-2 antisense oligonucleotides: a potential novel strategy for the treatment of breast cancer. Semin Oncol. 2003;30(5s16):143-9.
52. Rudin CM, Kozloff M, Hoffman PC, et al. Phase I study of G3139, a bcl-2 antisense oligonucleotide, combined with carboplatin and etoposidein patients with small-cell lung cancer. J Clin Oncol. 2004;22(6): 1110-7.
53. Akino K, Ohtsuru A, Yano H, et al. Antisense inhibition of parathyroid

hormone-related peptide gene expression reduces malignant pituitary tumor progression and metastases in the rat. Cancer Res. 1996; 56(1):77-86.
54. Qiu G, Goodchild J, Humphreys R, et al. Cancer immunotherapy by antisense suppression of Ii protein in MHC-II positive tumor cells. Cancer Immunol Immunother. 1999;48(9):499-506.
55. Ahmad S, Glazer RI. Expression of the antisense cDNA for protein kinase C alpha attenuates resistance in doxorubicin-resistant MCF-7 breast carcinoma cells. Mol Pharmacol. 1993;43(6):858-62.
56. Chan PR, Glazer RM. Triplex DNA: Fundamentals, advances and potential applications for gene therapy. J Mol Med. 1997;75:267-82.
57. Vasquez KM, Wilson IH. Triplex directed modification of genes and gene activity. Trends Biochem Sci. 1998;23:4-9.
58. Mayfield C, Ebbinghaus S, Gee I, et al. Triplex formation by the human Ha ras promoter inhibits Spl binding and in vitro transcription. J Biol Chem. 1994; 69:18232-8.
59. Alunni Fabbroni M, Pirolli D, Manzini G, et al. (A,G) oligonucleotides form extraordinary stable triple helices with a critical R.Y sequence of the murine c Ki ras promoter and inhibit transcription in transfected NIH 3T3 cells. Biochem. 1996;35:1636- 9.
60. Ebbinghaus S, Gee J, Rodu B, et al. Triplex formation inhibits HER 2/ neu transcription in vitro. J Clin Invest. 1993;92:2433-9.
61. Porumb H, Gousset H, Letellier R, et al. Temporary ex vivo inhibition of the expression of the human oncogene HER2 (NEU) by a triple helix forming oligonuclectide. Cancer Res. 1996;56:515-22.
62. Aggarwal B, Schwarz L, Hogan M, et al. Triple helix forming oligodeoxyribonucleotides targeted to the human tumor necrosis factor (TNF) gene inhibit TNF production and block the TNF dependent growth of human glioblastoma tumor cells. Cancer Res. 1996;56:5156-64.
63. Quintero G, Restrepo C, Abuchaibe O, et al. Cancer immunogene therapy: Antisense and Triple helix approach. Biomed J Sci Tech Res (BJSTR). 2018;2(3):doi: 10.26717/BJSTR.2018.03.000919.
64. Scaggiante B, Morassutti C, Tolazzi G, et al. Effect of unmodified triple helix forming oligodeoxyribonucleotide targeted to human multidrug resistance gene mdrl in MDR cancer cells. FEBS Lett. 1994;352:380-4.
65. Thomas T, Faaland C, Gallo M, et al. Suppression of c myc oncogene expression by a polyamine complexed triplex forming oligonucleotide in MCF 7 breast cancer cells. Nucleic Acids Res. 1995; 23: 3594-9.
66. Sharp P A. RNA interference–2001. Genes Dev. 2001;15:485 –90.
67. Dias N, Stein CA. Basic concepts and antisense oligonucleotides mechanism. Mol Cancer Ther. 2002; 1: 347-355.
68. Trojan J, Kopinski P, Drewa T, et al. Immunogenotherapy of prostate cancer. Urol. Pol. (Centr Eur J Urol). 2003; 56(2):7-11.
69. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators (8 October 2016). "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015". Lancet. 2016;388(10053): 1545–602.
70. Popiela T, Sierzega M, Gach T, et al. Phase I trial of colorectal cancer immunotherapy using autologous cancer cells transfected with an IGF-I antisense plasmid. Acta Chir Belg. 2003; 5(103): 2-3.
71. Trojan J, Ly A, Wei MX, et al. Antisense antii IGF-I cellular therapy of malignant tumours: immune response in cancer patients. Biomed Pharmacother. 2010;64:576-8.
72. Wongkajornsilp A, Ouyprasertkul M, Sangruchi T, et al. The analysis of peri-tumour necrosis following the subcutaneous implantation of autologous tumor cells transfected with an episome transcribing an antisense IGF-I RNA in a glioblastoma multiforme subject. J Med Assoc Thai. 2001;4(3):740-7.
73. Anderson WF, Blaese RM, Culver K. The ADA human gene therapy clinical protocol: Points to consider response with clinical protocol. Hum Gene Ther. 1990;1(3):331-62.
74. Ferrari G, Rossini S, Nobili N, et al. Transfer of the ADA gene into human ADA-deficient T lymphocytes reconstituites specific immune functionbs. Blood. 1992; 80(5): 1120-4.
75. Walther W, Stein US. Gene therapy of cancer. Methods and protocols. Ed. Human Press, NY, 2009.
76. Templeton NS. Gene and cell therapies. Ed. CRC Press, Fl, 2009.
77. You Y. Targets in gene therapy. Ed. InTech, Vienna, Riyeka, 2011.
78. Lichtor T. Evolution of the molecular biology of brain tumors and the therapeutic implications. Ed. InTech, Vienna, Riyeka, 2013.
79. Townsend SE, Allison JA. Tumor rejection after direct costi-mulation of CD8+ T cell transfected melanoma cells. Science. 1993; 259: 368-70.
80. 80. Guo Y, Wu M, Chen H, et al. Effective tumor vaccine generated by fusion of hepatoma cells with lymphocytes B cells. Science. 1994;263:518-20.
81. Zumkeller W, Westphal M. The IGF/IGFBP system in CNS malignancy. Mol Pathol. 2001:54; 227-9.
82. Kleihues P, Luis DN, Scheithauer BW et al. The WHO classification of tumors of the nervous system. J Neuropathol & Exp Neurol. 2002;(61)3: 215-25.
83. Harding BN, Golden JA. Developmental neuropathology. International Society of Neuropathology, Basel, Suiss. 2004.
84. Ohgaki H, Dessen P, Jourde B, et al. Genetic pathways to glioblastoma: A population-based study. Cancer Res. 2004;64(19):6892–9. doi: 10.1158/0008-5472.CAN-04-1337.
85. Guha A, Mukherjee J. Advances in the biology of astrocytomas. Current Opinion Neurol. 2004;17(6):655-62.
86. Wrensch M, Rice T, Miike R, et al. Diagnostic, treatment, and demographic factors influencing survival in a population-based study of adult glioma patients in the San Francisco Bay Area. Neuro Oncol. 2006;8(1):12-26. doi: 10.1215/S1522851705000268.
87. Esiri M, Perl D. Oppenheimer's diagnostic neuropathology. Ed. CRC Press, 3rd ed, FL, 2006.
88. Stupp R, Hegi ME, van den Bent MJ, et al. Changing paradigms--an update on the multidisciplinary management of malignant glioma. Oncologist. 2006;11:165-80.
89. Love S, Arie Perry A, Ironside J, et al. Greenfield's Neuropathology. CRC Press, 9th ed, NY, 2015.
90. Benedetti E, Galzio R, D'Angelo B, et al. PPARs in human neuroepithelial tumors: PPAR ligands as anticancer therapies for the most common human neuroepithelial tumors. PPAR Res. 2010;401-427.
91. Le Gall T, Loizeau D, Picquet E, et al. (2010). A novel cationic lipophosphoramide with diunsaturated lipid chain synthesis:physicochemical properties and transfection activities. J Med Chem. 2010;53:1496-508.
92. Costa PM, Cardoso AL, Mendonça LS, et al. Tumor-targeted chlorotoxin-coupled nanoparticles for nucleic acid delivery to glioblastoma cells: a promising system for glioblastoma treatment. Mol Ther Nucleic Acids. 2013;2:e100.doi:10.1038/mtna.2013.30.
93. Zhang J, Han L, Zhang A, et al. AKT2 expression is associated with glioma malignant progression and required for cell survival and invasion. Oncology Report. 2010;24(1): 65-72.
94. Le Corre SS, Berchel M, Belmadi N, et al. Cationic lipophosforamidates with two different lipid chains: synthesis and evaluation as gene carriers. Organic & Biomol Chem. 2014;2:1463-74.
95. Le Roith D, Bondy C, Yakar S, et al. The somatomedin hypothesis. Endocrinol Rev. 2011; 22(1): 53-74.
96. Ertl DA, Gleiss A, Sagmeister S, et al. Determining the normal range for IGF-I, IGFBP-3, and ALS: New reference data based on current internal standards. Wiener Med Wochenschr. 2014;164 (17-18):343-52.

97. Hu B, Niu X, Cheng L, et al. Discovering cancer biomarkers from clinical samples by protein microarrays. Proteomics Clinical Application. 2015;9(1–2):98-110.
98. Pan Q, Luo X, Chegini N. Blocking neuropilin-1 function has an additive effect with anti VEGF to inhibit tumor growth. Cancer Cell. 2007;11(1): 53-67.
99. Zhou X, Ren Y, Moore L, et al. Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status. Lab Investig. 2010;90:144-155.
100. Sachdev D. Targeting the type I Insulin-like Growth Factor system for breast cancer therapy. Curr Drug Targets. 2010;11:1121-32.
101. Yang L, Lin Z, Huang Q, Effect of vascular endothelial growth factor on remodeling of C6 glioma tissue in vivo. J Neuro-Oncol. 2011;103(1), 33-41.
102. Chen H, Mester T, Raychaudhuri N, et al. Teprotumumab, an IGF-1R blocking monoclonal antibodyinhibits TSH and IGF-1 action in fibrocytes. J Clin Endocrinol Metab. 2014;99(9):E1635-1640.
103. Bueno S, Santander R, Alvarez A, et al. Brain stem cells and IGFI: Implications in development, regeneration and cancer therapeutics. Integr Mol Med. 2018;5(1); DOI: 10.15761/IMM.1000319
104. Hutterer M, Gunsilius E, Stockhammer G. Molecular therapies for malignan glioma. Wiener Med Wochenschrift, 2006;156(11-12), 351-63.
105. Choi BD, Archer GE, Mitchell DA, et al. EGFRvIII-targeted vaccination therapy of malignant glioma. Brain Pathol. 2009;19(4) :713-23.
106. Li Y, Jia Q, Zhang J, et al. Combination therapy with Gamma Knife radiosurgery and antisense EGFR for malignant glioma in vitro and orthotopic xenografts. Oncol Report. 2010;23(6): 1585-91.
107. Kalman B, Szep E, Garzuly F, et al.. Epidermal growth factor receptor as a herapeutic target in glioblastoma. Neuromol Med. 2013;15(2):420-34.
108. Gaffney DC, Soyer HP, Simpson F. The epidermal growth factor receptor in squamous cell carcinoma: An emerging drug target. Australasian J Dermatol. 2014;55(1):24-34.
109. Lo HW. Targeting Ras-RAF-ERK and its interactive pathways as a novel therapy for malignant gliomas. Curr Cancer Drug Targets. 2010;10:840-8.
110. Hau, P, Jachimczak P, Schlaier J, et al. TGF-β2 signaling in high-grade gliomas. Curr Pharmacol Biotechnol. 2011;12:2150-7.
111. Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4 and B7/BBI in interleukin-2 production and immunotherapy. Cell. 1992;71:1065-8.
112. Blanchet O, Bourge JF, Zinszner H, et al. Altered binding of regulatory factors to HLA class I enhancer sequence in human tumor cell lines lacking class I antigen expression. Proc Natl Acad Sci USA. 1992;89(8):3488-349.
113. Boado RJ. RNA interference and nonviral targeted gene therapy of experimental brain cancer. NeuroRx. 2005;2(1):139-50.
114. Berezikov E, Thuemmler F, van Laake LW, et al. Diversity of microRNAs in human and chimpanzee brain. Nature Genetics. 2006;38(12):1375-7.
115. Corsten MF, Miranda R, Kasmieh R, et al. MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. Cancer Res. 2007;67(19):8994-9000.
116. Hegi M E, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. New Engl J Med. 2005;352:997-1003.
117. Zhu C, Trabado S, Fan Y, et al. Characterization of effector components from the humoral and cellular immune response stimulated by melanoma cells exhibiting modified IGF-1 expression. Biomed & Pharmacother: 2015;70:53-7.
118. Trojan J. Brain - from development to neoplasia and gene therapy solution. Ed. Lambert Academic Publishers, Germany, 2017/2018; pp 1 – 150, ISBN : 978-620-2-08024-8.
119. Hajeri-Germond M, Naval J, Trojan J et al. The uptake of alphafetoprotein by C-1300 mouse neuroblastoma cells. Brit J Cancer Res. 1985;51:791-7.

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