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## Research article

## Twenty-Five Years of Cancer Gene 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 derivated of all three embryonic layers; in the case of tumor derivated 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 sys-

tem 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].

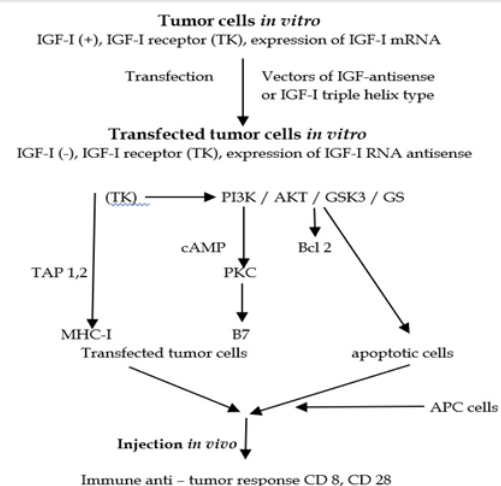
The results obtained so far with classical treatment of malignant tumors, especially of glioblastoma (surgery and chemotherapy) are unsatisfactory, as the survival of these recurrent patients is discouraging low. In order to find a specific solution for cancer treatment, the purpose of our strategy was 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 (Vitravene™) (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, TGFβ, EGF, VEGF [9,26,45-47]; antisense oncogenes i.e. c-myc, bcr/abl, ras [43,48-50]; antisense of apoptosis related elements i.e. bcl-2 [51,52]; antisense of pro-

tein 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, TNFα, EGFR [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 derivated 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 cultured in 20% bovine serum in DMEM/F12. Three million cells were seeded per well in gelatin-covered 6 well plates. Using antisense/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 adenocarcinoma or colon adenocarcinoma. 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/GW3/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 adenocarcinoma	5 weeks	4 weeks	C-26% V1-21% V2-20%	C-12% V1-24% V2-25%
K.S.	53	prostate adenocarcinoma	5 weeks	5 weeks	C-25% V1-22% V2-20%	C-13% V1-24% V2-27%
B.K.	57	ovarian carcinoma	4 weeks	4 weeks	C-24% V1-20% V2-19%	C-13% V1-22% V2-26%
B.H.	50	ovarian carcinoma	4 weeks	3weeks	C-26% V1-22% V2-19%	C-15% V1-24% V2-28%
A.M.	56	colon adenocarcinoma	5 weeks	5 weeks	C-26% V1-25% V2-21%	C-10% V1-25% V2-30%
S.K.	48	colon adenocarcinoma	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 interfering 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 oligodesoxynucleotides 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.

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