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Anti – Gene IGF-I Technology applied for Cancer Immunotherapy

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***Abstract*—IGF-I is one of the most important growth factors related to normal differentiation, and its overproduction in mature tissues is a sign of neoplastic processesin three derivatives: neuroectodermal e.g. brain malignant glioma, entodermal e.g. hepatocarcinoma, and mesodermal e.g. prostate adenocarcinoma. The creation in the early 1990s of a new domain in cancerology named cancer immunogene therapy, has revolutionized the treatment of tumors expressing IGF-I. This new strategy, using anti-gene, either antisense or triple helix anti IGF-I technology, has shown promising results in clinical trials; the median survival of glioblastoma patients reached 21 months and in some cases, three to four years. This strategy was also proven to be efficient in the treatment of liver and prostate cancers.**

***Index Terms*—antisense, cancer, IGF-I, immunotherapy**

I. Introduction

A. General view

The treatment of malignant tumors using surgery, radiation, hormonetherapy and chemotherapy are limited in their effectiveness. Therefore, new forms of treatment have become a mandatory challenge; in the case of glioblastoma, the average survival rate is only 10-14 months. Clinical cancer research has not found a solution for the treatment of this incurable disease,yet basic cancer research using immunology and molecular biology knowledge has proposed promising possibilities. Cell immunotherapy or cell immunogenetherapy of cancer is one of the latest approaches to the treatment of various forms of malignant tumors. Since 2015, USAcancer "Moonshot" program (http://www.cancermoonshot2020.org/about-us/), has underlined the importance of cancer immunotherapy [1-5].

Our strategy of cancer immunotherapy was established as immunogenetherapy targeting*IGF-I gene* in the tumors of three derivatives: neuroectodermal – i.e. glioblastoma, as endodermal – i.e. hepatocarcinoma, and mesodermal – i.e. prostate adenocarcinoma [1, 6].

Brain malignant tumor - Glioblastoma multiforme (GBM) represents about 45-50% of intracranial tumors.

GBM, a primitive brain tumor, has an annual incidence ranging between 2-19 /100,000. Gliobastoma has a very poor prognosis. The standard treatment of primary glioblastoma is surgery followed by radiation therapy combining temozolomide-based chemotherapy. The median survival is only 12-15 months.

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Liver cancer - hepatocellular carcinoma or hepatocarcinoma (HCC) represents 80-90% of the primary malignant liver tumors. Its incidence is at a gross rate of 11 / 100,000. This tumor is due to liver cirrhosis in about 90% of cases. Until very recently, there was no treatment for this disease. Different treatments are now available: surgical resection, liver transplantation, chemoembolization -TACE, sorafenib.

Prostate cancer - prostate adenocarcinoma, is ranked second in cancer mortality in men. The age-adjusted rate of cancer mortality was 12 / 1000.000 men. The risk factor is a diet of higher fat intake. The prognosis related to prostate cancer is relatively good. Treatment may include surgery, radiation, chemotherapy, or a combination of all.

B. Challenge

We have been facing a real challenge when it comes to the treatment of malignant tumors, and more specifically, glioblastoma. Which tools should be developed and applied at a clinical level - using our knowledge of evolution, oncodevelopmental biology, neuroscience, genetics, chemistry, molecular biology and immunology. In the years 1970-1980, what we knew about the brain, its normal and neoplastic development, function and cancer treatment was scarce. The first step was studying the normal development of the central nervous system, and comparing it with neoplastic brain development, as well as studying other tissue derivatives, and more specifically normal and neoplastic entodermal tissues – normal and cancerous liver. Between 1979 and 1984, through the use of a new marker, alpha-fetoprotein, AFP [7,8], a first description of the development of the central nervous system and liver using AFP marker was published [9]. Using the same marker and investigating the neoplastic development of nervous system and also the neoplastic endodermal and mesodermal derivatives using mouse teratocarcinoma model, studies showed that there was a convergence between embryo/fetal development and neoplastic development [10]. This discovery facilitated research on neoplastic development of brain, liver and prostate.

When considering AFP marker of neoplastic development, this oncoprotein is present not only in glial, but also in neuronal neoplastic cells. Another oncoprotein, growth factor IGF-I, was proposed to study brain glial malignant tumors. IGF-I is a marker of glial neoplastic cells (and also of liver and prostate neoplastic cells), and is absent in

neuronal neoplastic cells [11-15]. This observation was decisive to distinguish glial and neuronal differentiation, and to target IGF-I in glial malignant tumor – glioblastoma. Moreover, IGF-I is considered as the most important growth factor of normal and neoplastic development [16-21].
Knowing that the strategy targeting oncoproteins using antibodies has not been effective in the treatment of cancers, another possibility was explored: stopping the synthesis of oncoproteins at translation or transcription levels. The arrest of synthesis at translation level was done using antisense technology [22-24 (Rubenstein et al, 1984; Weintraub et al, 1985; Green et al, 1986)] with a vector expressing antisense IGF–I RNA [1,25, 26 (Trojan et al, 1992 and 1993; Ellouk-Achard et al, 1998)]. This approach has shown positive *in vitro* results – arrest of IGF-I synthesis in cancer cells, and also *in vivo* - stopping the tumor development. These two *in vitro* and *in vivo*effects, have given birth to cancer gene therapy [27]. The efficiency of molecular biology techniques in suppression of IGF-I oncoprotein expression using antisens technology was confirmed by another technique stopping the oncoprotein synthesis at transcription level – triple helix approach [28, 29] demonstrated in glial and liver cancer cells [30, 31].

. II. METHODOLOGY

An efficient strategy was established by construction of vectors targeting growth factors present in neoplastic development. Anti-Gene technology was applied to construct the vectors expressing either IGF-I antisense RNA or IGF-I RNA forming RNA-DNA triple helix [25,30]. The vectors introduced in the cells in vitro, enable to completely stop the synthesis of growth factor on translation or transcription level, respectively. When injected in vivo in animals bearing tumors or applied in clinical treatment of glioblastoma patients, these genetically modified cells induce an immune anti-tumor effect (CD8+) accompanied by increase of the median survival of patients (successful clinical results obtained in USA, EU and China) [1,6,14,31-33].

A. Anti – Gene technology

Currently the "anti-gene" strategies offer new possibilities for cancer therapy and among them, "antisense" and "triple helix" techniques seem very promising, stopping the protein synthesis on transcription level [24], and translation level [28], respectively. The principal strategies of gene therapy for treatment of gliomas, and also liver cancers, including antisense approach, have been proposed since the 1990s [1,25,34-36].

B. Antisense approach

The "discovery" of antisense approach was done by the groups of F. Jacob and R.M. Harland [22,23]. This event has been suggested to physiologically occur as the regulation mechanism of gene expression in cells. It has been widely proven that a lot of genes present an open reading frame on the antisense strand [37-39]. Concerning natural antisense RNA in prokaryotes, it has been shown that they could play a regulatory role in replication, transcription or translation steps of some genes [23].
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 [22] 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. The antisense oligodeoxynucleotides, once in the cell, can stimulate the ribonuclease H after hybridization with target RNA. This enzyme, which is implicated in DNA replication, damages RNA moiety of the hybrids formed in the cell [29]. 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[40].

The first antisense oligonucleotide used in clinical pharmacology was as anti-cytomegalovirus therapy [41]. The antisense strategy was then largely used in order to analyze gene expression and intron splicing. The antisense technology was used to study several protein actions: the alpha subunit of human chorionic gonadotropin in choriocarcinoma cells [42]; the regulating protein E2F-1, in S cellular cycle phase, and its action on genes linked to proliferation [43]; nerve growth factor (NGF) in skin of transgenic mice, and its relationship with response to mechanical stimuli [441.

In "antisense" anti-tumor therapy different strategies were applied. Among them are strategies based generally on antisense of:
genes encoding growth factors [1] ,
genes encoding enzymes [45],
oncogenes [46],
proteins related to MHC expression [47],
elements of signal transduction [48],
inhibitor of apoptosis [49],
miR, i.e. miR-21, -92, -143, -145, -221/222 [50],
heat schock-protein [51],
laminin, i.e. -411 [52],
metallo-thionein [53],
The antisense therapy is especially useful in research and clinical studies concerning human malignancies [54-56].

It was showed that c-myb antisense phosphorothioate (S) oligodeoxynucleotides treatment decreases c-myb mRNA and protein expression [57]. The study suggests that c-myb antisense (S) oligodeoxynucleotides might be useful in the therapy of cancers in combination with chemotherapeutic drugs.

Antisense strategy was also used in leukemia and lymphoma treatment to eliminate malignant blood cells. The anti-bcr/abl antisense was applied to purify a patient's bone marrow [40]

The ras family belongs to the most frequently activated oncogenes in human cancer and is widely used as the

triple-helix strategy target. It was demonstrated that triple helix forming oligonucleotides could inhibit the transcription of murine Ki-rasgene in tumor cells [58]. Antisense oligonucleotide-targeted K-ras gene may be a good choice for therapy because it could inhibit the growth in of 5-FU and mitomycin C resistant metastatic and remetastatic cells as well as in primary tumor cell [59,60].

The diminished apoptotic response caused by bcl-2 overexpression is associated with IGF-I presence [32,61]. and cellular resistance to chemotherapeutic drugs; downregulation of bcl-2 by antisense oligonucleotides has been shown to improve the efficacy of chemotherapy in phase III randomized clinical trials in patients with solid tumors [62]. bcl-2 is also expressed in the majority of cases of small cell lung cancer (SCLC) and may contribute to chemotherapeutic resistance. bcl-2 suppression by G3139 (antisense oligodeoxynucleotide targeted bcl-2 mRNA open reading frame) has the potential to enhance the antitumor efficacy of standard cytotoxic chemotherapy[63].

TGF-beta, which is expressed by a majority of malignant tumors, is the most potent immunosuppressor and in addition stimulates angiogenesis. Reversal of TGF-beta-induced immunosuppression is a new and promising approach to cancer therapy using antisense approach [64-67].

The most extensively studied receptor in the erbB family is the human epidermal growth factor receptor (EGFR), also known as erbB1. Studies have shown that overexpression of EGFR is involved in the development and progression of head and neck squamous cell carcinoma (HNSCC). Preliminary results from early phase clinical trials using antisense EGFR are encouraging [68-70].

The vascular-endothelial growth factor (VEGF) is an endothelial mitogen factor inducing angiogenesis in solid tumors. It was determined that VEGF antisense oligonucleotide treatment can decrease angiogenic activity [71-73]. VEGF antisense oligodeoxynucleotides inhibit VEGF expression of liver cancer cells. VEGF antisense oligodeoxynucleotides are essentially efficient if mixed with lipiodol embolizing liver cancer [74,75].

The expression of PTHrP peptide synthetized by pituitary metastatic tumor cells was suppressed in the tumoral cells transfected with antisense oligonucleotides of PTHrP. Thus, the application of antisense technology could be a plausible strategy for the treatment of metastases of somatotropic tumors [76]. IGF-I being related to neoplastic differentiation [17], IGF-I antisense gene therapy was proposed to treat glioma and hepatic cancer [1,6], and then successfully applied in clinical trials [77].

C. Triple helix approach

The triple helix (TH) technology belongs together with antisense approach to anti-gene strategies sensu lato, i.e. the technics targeting the expression of respective up-regulated gene. The TH technology was “discovered” by groups of P.B. Derwan[28] and of C. Helene [29]. Its action is well 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[28]. This TFO generated in situ is therefore protected from degradation by nucleases and could reach its DNA target without being trapped in lysosomal vesicles. An application of this triplex-based approach has been used for the inhibition of the IGF-I which plays a major role in tumorigenesis [30]. The examples of the inhibitory activity of triplex-forming oligonucleotides on target genes involved in tumorigenesis are now available [78,79].

Triple helix strategy coming to be successfully introduced in experimental and clinical gene therapy trials [80]. Triple helix strategy was applied to the ras oncogenes which are the most frequently activated oncogenes in human cancer. In vitro transcription of human Ha-ras was inhibited by triplex-forming oligonucleotides [58].

The synthesis of human tumor necrosis factor (TNF), which acts as an autocrine growth factor in various malignant tumors including glioblastoma, has been blocked by triplex-forming oligonucleotide treatment [81]. Triplex-forming oligonocleotides were also shown to bind *in vitro* to human EGF receptor promoter, and to inhibit transcription of HER2/neu gene which is overexpressed in breast cancer and other human malignancies[82].

A novel, and potentially remarkable, development in oligonucleotidetechnology is the relatively recent finding that 21–23-merdouble-stranded RNA molecules, known as siRNA, can effectivelysilence gene expression [83]. The role of 22-23 mer RNA in silencing of gene is strongly similar to that of triple helix RNA-DNA mechanism involving also 23 mer RNA[29,84].

D. Clinical trial

First of all, the selected patients have presented diagnostically confirmed astrocytoma IV (glioblastoma), or hepatocarcinoma or prostat 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).

The first obligatory treatment of selected patients was surgery done according to the classical protocol for glioblastoma and liver and prostate cancers 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 (six sessions of radiation). During this period of radiotherapy the patients were treated also with chemotherapy using a low dose of temozolomide (glioblastoma). Temozolomide dosing regimen, oral dose, were applied in newly diagnosed glioma,

as 75 mg / m2 per day for 42 days. The drug was ingested one hour before radiotherapy. The radiotherapy was followed by immunotherapy without chemotherapy (1st group): (1) four subcutaneous vaccinations with autologous AS/TH was performed with interval of one month. In the 2nd group, the radiotherapy was followed by the same low dose classical chemotherapy [85,86].

For clinical protocol, every group (the first with immunotherapy, and second without immunotherapy) as well of glioblastoma patients as of liver and prostate cancer patients, was composed of four patients (17 – 70 years old) were treated. As to liver and prostate cancers, the patients have followed classical protocols. No radiotherapy and no chemotherapy was applied after surgery. Two months after surgery, the immunotherapy was introduced: four subcutaneous vaccinations with autologous AS/TH was performed with interval of one month (1st group). The 2nd control group was not treated by immunotherapy. [86].

E. 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-centimeter diameter biopsies were placed in DMEM+F12 medium containing high glucose concentration. Specimen were then transferred to PBS with no Ca++ and Mg++ and dissected into 2-mm pieces. PBS were changed to PBS containing collagenase. The tissue was then 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 and incubated [87].

Using antisense /triple helix IGF-I expressing vectors (50:50), transfection was done during 3-4 weeks, by either Ca++/Ph technique or FuGENE 6 Transfection Reagent (Boehringer Mannheim). 48 hours after transfection, the selection of transfected cells was done in the presence of Hygromycin B (Boehringer Mannheim) at a concentration of 0,005 mg/ml. After one week, concentration of hygromycin B will be changed to 0,015 mg/ml, and progressively increased up to 0,15 mg/ml. Two weeks after transfection, cell lines derived of the same tumor, were verified for absence of IGF-I (using immunocytochemistry technique, confirmed by RT PCR technique), and for presence of MHC-I molecules using flow cytometry analysis (monoclonal antibodies, labeling human MHC-I (HLA), MHC-II, CD80 and CD86 antigens, provided by Becton Dickinson Pharmingen). 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(+) [86].

F. Cellular immunotherapy

The celular immunotherapy was done applying three subcutaneous injections into left arm (1 ml of physiologic solution containing 1,5 millions of transfected cancer cells with anti - IGF-I antisense/ triple helix vectors). The interval of one month was applied between three succesive injections. 48 hours before every injection, the cells were irradiated with 5000 cGy gamma (Co60 or Cs137).

The blood samples were removed before injection, and then three weeks after every injection. The PBL cells were examined by flow cytometer analysis for verification of immune anti-tumor response. The following markers were considered: CD8 / CD4, CD8+11b+ / CD8+11b-, CD 28 [77].

The patent related to Anti – Gene anti IGF-I cellular Immunotherapy procedure was registered previously (Gene therapy of tumours expressing IGF-I PICB, No 6312 « Passeport Intellectuel Copyright Business» USD System inc., Paris, France & Montreal, Canada, 1999 and 2005).

III. RESULTS

A. Clinical 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 are 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 (**Fig. 1**).

The first clinical trial concerned glioblastoma patients [93,94] followed by liver and prostate cancers patients [86]. In our clinical trials, two glioblastoma patients included in "cellular therapy" group (1st group) have survived 19 and 24 months, respectively. Two glioblastoma patients included in the 2nd group, without immunotherapy, have survived about 9,5 and 10 months, respectively – coming from surgery followed by radiotherapy only.

The results observed in the 2nd group of glioblastoma patients, not treated with "cellular therapy", were not so different from those obtained using a classical treatment composed of surgery, radiotherapy and chemotherapy (high dose of temozolomid 150 -200 mg/m2 per day x 5 days, every 28 days, applied for 6 months), median survival being as 10 – 11 months, rarely 13 months.

Admitting that 1st group of glioblastoma treated patients, using immunotherapy, has given spectacular results, all liver and prostate cancer patients treated with this type of "cellular therapy" (1st group) were supervised clinically up to two years, including the control 2nd group.

At 19 months, all liver and prostate cancer patients treated by surgery followed by cellular immunotherapy were alive and the treatments were well tolerated. The only secondary effect observed in treated liver and prostate cancer and also in 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 [77,86]. No other secondary effects were registered.

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-, confirming the effectiveness of "cellular

therapy". 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 as well in glioma as in liver and prostate cancer treated patients (**Fig. 2**). The results concerning other studied CD molecules as CD 3, CD19, CD45 (data not shown) were non significant in all cancer treated patients; in the case of CD4 slightly decreased values were registered.

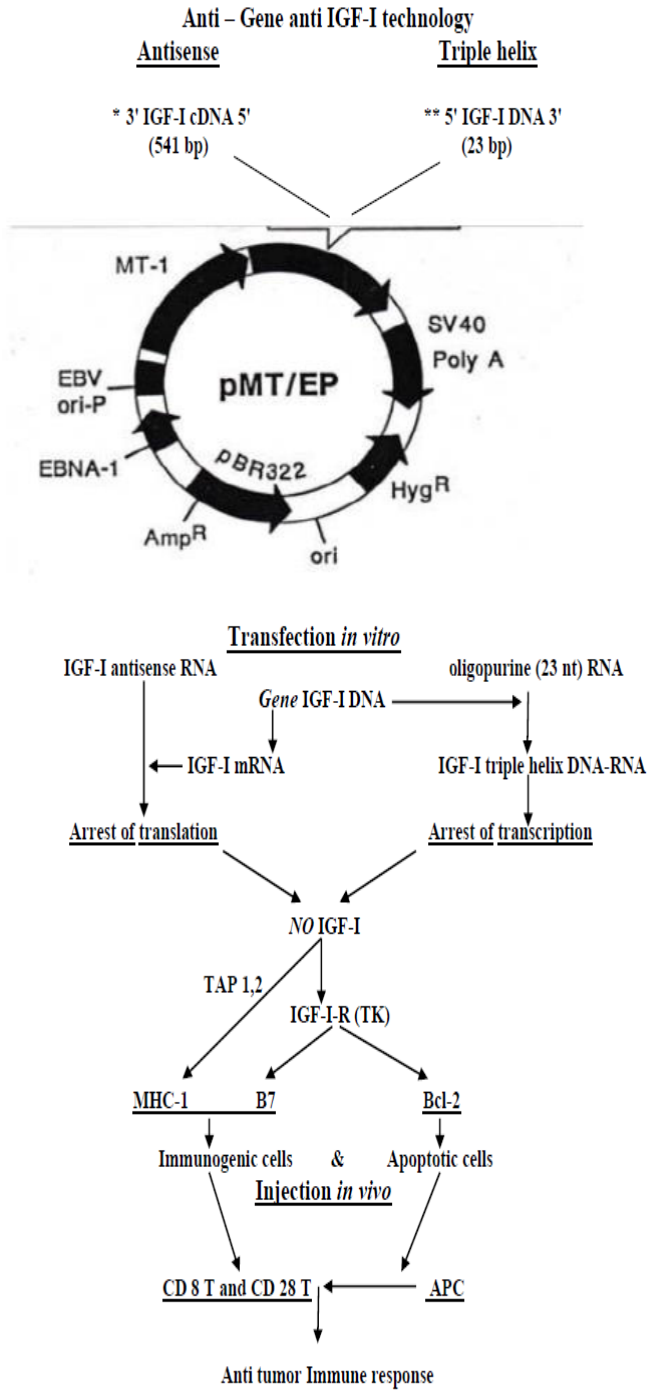

Fig. 1. Anti – Gene immunogene therapy. Antisene anti IGF-I and Triple Helix anti IGF-I expression vectors were prepared using pMT/EP "empty" vector [25,30]. * fragment of IGF-I cDNA in antisense orientation inserted in Multiple cloning sites, MCS, of empty vector which transcribes an antisense RNA. ** 23 bp sequence prepared by PCR, inserted in MCS of empty vector which transcribes an RNA forming a triple helix structure (Hoogsteen bonds) within the target region of the human 1st promoter of IGF-I gene. Amp^R – ampicilin resistance gene; EBNA-I and EBV ori – Epstein Barr Virus encoded Nuclear Antigen I; MT-I - metallothionein I promoter; SV 40 poly A – simian virus 40 poly(A) addition; Hyg^R – hygromycin resistance gene; pBR322 ori – plasmid derivatived of pBR322.

After transfection *in vitro* of tumor cells with any of both types of vectors, the synthesis of IGF-I is stopped, and the cells express MHC-I, mediated by TAP1 and 2, and B7 mediated by signal transduction pathway of IGF-I receptor (tyrosine kinase). These cells in part enter in apoptosis, mediated by Bcl2. The injection of immunogenic and apoptotic cells (50:50) in cancer patients has induced in the presence of APC cells, the cell immune response (T CD8 and T CD 28) [88-92].

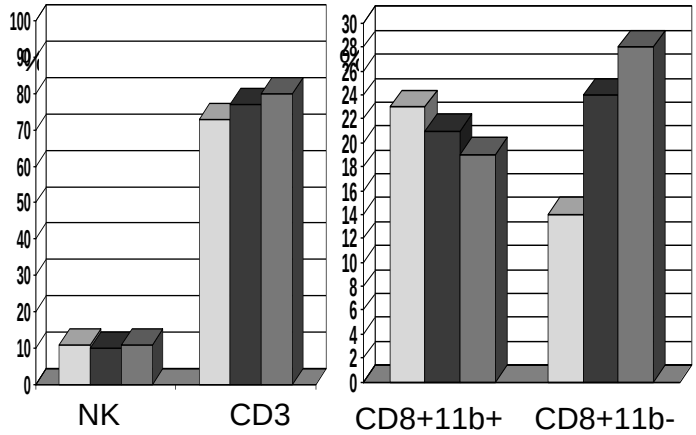

Fig. 2a

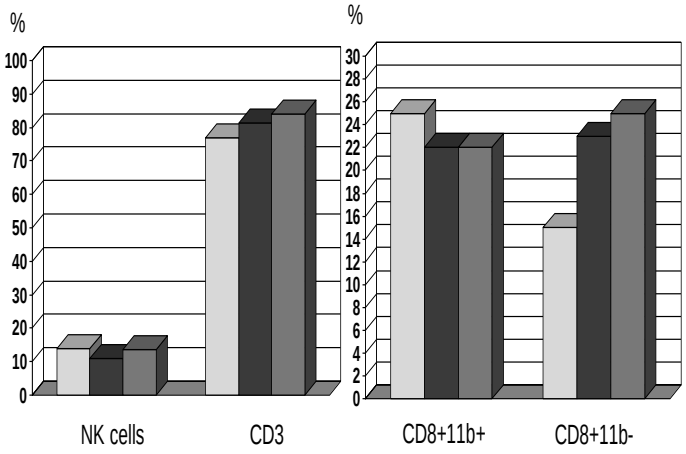

Fig. 2b

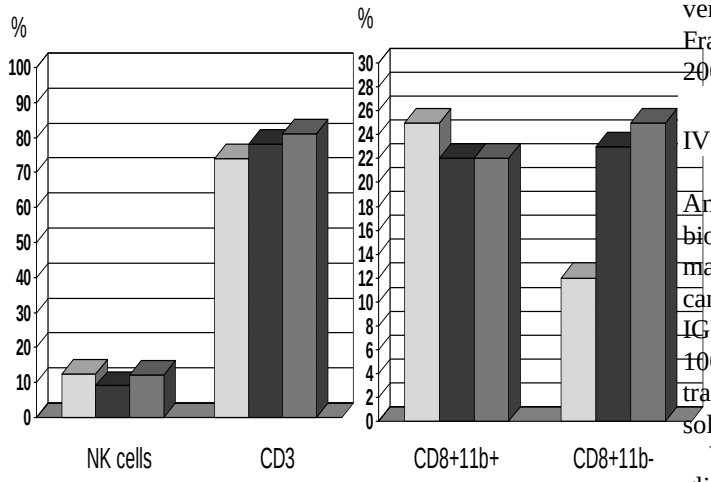

Fig. 2c

Fig. 2a, b, c. Antisense/Triple helix anti IGF-I cellular gene therapy of cancer patients: a – glioblastoma, b – hepatocarcinoma, c – prostate adenocarcinoma. Expression of CD molecules in peripheral blood lymphocytes (PBL) derived from "vaccinated" cancer patients: all studied antigens are mentioned as NK, CD19, CD4, CD8, CD8+11b+ and 11b-. Every first column corresponds to data obtained before vaccination; every second and third column corresponds to data obtained after 1st and after 2nd vaccination, respectively. Two cases of each cancer disease were examined (every column represent the median value of two cases). The first vaccination was done using the injection of cell membranes derivated from 1-2 x 105 antisense IGF-I cells, followed three weeks later by the injection of 1,5 – 2 x 10 6 antisense IGF-I cells. The second vaccination was done one month later by the injection of the same quantity of antisense cells. PBL were analyzed by flow cytometry analysis (FACScan Becton Dickinson): double direct immunotyping with pairs of monoclonal antibodies conjugated with FITC and PE, respectively. Lymphocyte gate was defined according to the CD45 backgating. Data are expressed as percent of positive cells as compared to the isotype control [77,86].

B. Ethical Committees

Both IGF-I anti-gene therapies: IGF-I antisense and IGF-I triple helix, were introduced in clinical trials as follows (see chapter – Ethical Committees): glioblastoma (Cleveland, USA; Bangkok, Thailand; Bromberg, Poland – collaboration with Paris, France), liver hepatocarcinoma (Shanghai, China, and Cracow, Poland), prostate, ovary, uterus and colon cancers (Bromberg, Poland in collaboration with Paris, France), and lung, stomach and epidermoid cancers (Bogota, Colombia - clinical study in progress).

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 (n˚ KB/176/2001, 28/06/2002, and n˚ 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, n˚ 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 (n˚ LST 980517).

IV. DISCUSSION

Among the new therapy strategies based on molecular biology and immunology techniques in efforts to treat malignant tumors especially glioblastoma, liver and prostate cancers [95-99], the approaches targeting growth factors as IGF-I, TGF-beta, VEGF or EGF [1, 21, 26, 66, 72, 73, 77, 100 -103], their receptors [68-70, 104, 105] and signal transduction elements [60, 106] seem to offer hope for a solution.

With current treatments, the survival of patients with glioblastoma is 15 months. Using Anti - Gene anti IGF-I methodology the median survival increased to 20-24 months. In some cases, the survival reached 3 or 4 years. As to hepatocarcinoma, the survival of patients treated with the same Anti IGF-I immunogene therapy, was 5 years (personal communication of Dr. Guo Yajun, 2nd Military Hospital of Shanghai). Recently the Anti - Gene anti IGF-I immunogene therapy was introduced in Colombia, and the first cases of treated lung and skin cancers have shown promising results [27]. Different malignancies including liver, prostate cancers and glioblastoma were recently successfully treated by antisense therapy focused on TGF-beta, using either a vector expressing antisense anti TGF beta [107] or in particular, applying oligodeoxynucleotides anti TGF-beta [66, 102, 108-110].

The "security''of methodology in genetic engineering is guaranteed by the use of an episomal vector, unlike other gene therapy techniques, which are based on retroviral vectors involving the theoretical risk of integration of the vector's DNA, which was designated by NIH Committee in USA [111], concerning the use of antisense therapy anti - IGF-I (episomal vector) [32,77]. In order to define new treatments, various techniques were investigated, among these the use of inhibitors [89]. Other technics include potentially useful siRNA (small RNA interferncia) [112,113] and miRNA (microRNA) [114]. The mechanism of siRNA in gene silencing is very similar to that of TH [29]. Currently the use of siRNA (small transfer RNA) has showed some problems: the siRNAs can ''omit'' the target involuntarily because it is structurally related to micro RNA. It can also result in non-specific events due to the activation of the innate immune response. As to miRNAs, they can play a key role in tumorigenesis, control of cell proliferation and apoptosis, and the use of miRNAs technology has shown some promising experimental results [115]. Whether or not siRNA or miRNA technologies can supplant the earlier mentioned approachesand their use of oligodeoxynucleotides remains in question at this time as we do not have final clinical results.

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 an immune anti-tumor response (CD8 +), and an inhibition of signal transduction pathway (PI3K / AKT / GWK3 / GS) involved in the tumor phenotype [67,87,116] (figure 2). The CD8 + cells can exert their cytotoxic effect, if they form a bridge with the MHC-I

antigen [92,117-120]. For this reason also, another strategy of glioblastoma treatment applying antisense anti IGF-I-Receptor technology has not given the expected results due to the absence of MHC-I expression in transfected cancer cells.

In conclusion, our article draws attention to recent studies in the field of cancer immunotherapy using the approach of Anti - Gene (AS, TH) technology alone, or combined with drug treatment [55,61,77, 93, 121].

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