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# Oncoproteins Targeting: Antibodies, Antisense, Triple-helix. Case of Anti IGF-I Cancer Immunogene Therapy

Jerzy Trojan

## Abstract

AFP and IGF-I oncoproteins were introduced as biomarkers for cancer diagnosis and targeted in cancer therapy on protein level, but also on transcription and translation levels. The protein level was targeted using an injection of antibodies or radiolabeled proteins. The transcription and translation levels were targeted by triple helix and antisense technologies, respectively. AFP was especially useful for diagnosis and therapy of liver cancer, IGF-I was applied in diagnosis and therapy of colon, prostate, liver, uterus, ovary and brain tumors. The most spectacular results were obtained with IGF-I anti-gene strategy. IGF-I antisense (AS)/triple helix (TH) gene therapy was successfully introduced in clinical trial in the USA and Europe. When using IGF-I anti-gene therapy, cancer cells provided from biopsies were transfected *in vitro* with IGF-I AS, IGF-I TH expression vectors. A decrease in IGF-I gene expression of 80 and 60% was demonstrated when using TH and AS technologies, respectively. These transfected cells expressing MHC-I molecules, while injected *in vivo*, induced immune antitumor response mediated by CD8 lymphocytes. The median survival of treated glioblastoma patients was 21–22 months. IGF-I AS/TH immunogene therapy constitutes one of the most promising approaches in cancer therapy, and more specifically when it comes to glioblastoma treatment.

**Keywords:** cancer, glioma, alpha-fetoprotein, IGF-I, diagnostic, antibodies, antisense, triple helix, immunotherapy

## 1. Introduction

Oncoproteins like alpha-fetoprotein (AFP), serum albumin (AS), and growth factors such as GH, IGF, TGF-beta, and EGF are present in embryo-fetal tissues and reappear in neoplastic developing tissues, including the central nervous system; this concerns especially AFP [1–8] and IGF-I [9–17]. AFP is present in both neural and glial developing and cancerous cells, whereas IGF-I is only present in glial developing and tumoral cells [10, 18]. This striking difference has helped us orientate a strategy to manage the most malignant brain tumor expressing IGF-I gene—glioblastoma.

IGF-I plays an important role in growth as a mediator of growth hormone [19–21]. Blocking IGF-I synthesis induces apoptotic and immunogenic phenomena [12, 22]. Both phenomena, apoptosis and immunogenicity, related to arrest of IGF-I expression

in neoplastic glial cells, were used to prepare antitumor cell vaccines for therapy of brain malignant tumor—glioblastoma [12, 23].

An efficient strategy targeting IGF-I was established by construction of vectors stopping the synthesis of this oncoprotein on translation and transcription levels: vectors expressing either IGF-I antisense RNA or IGF-I RNA forming RNA-DNA triple helix, respectively. The glioma cells transfected with these vectors, when injected *in vivo* in animals bearing tumors like glioma or teratocarcinoma or applied in clinical treatment of glioblastoma patients, induced an immune antitumor effect (CD8+) accompanied by increase of the median survival of patients (successful clinical results obtained in the USA, E.U., China) [22, 23].

## 2. Oncoproteins in diagnostic: AFP and IGF-I

### 2.1 AFP

AFP is present in normal and neoplastic developing tissues [1, 24–26]. The presence of AFP seems to be related to the stage of cell and tissue differentiation. AFP is absent from either undifferentiated or fully differentiated cells [24].

The localization of AFP was compared with that of another oncoprotein—serum albumin, SA. SA-mRNA gave a strong signal in differentiating structures as well as in undifferentiated cell clusters. AFP-mRNA was observed only in differentiating structures; this observation was especially useful in the clinical diagnostic of hepatocarcinoma [4, 27].

During an experiment with teratocarcinoma-bearing mice injected intraperitoneally with J-125 radiolabeled SA and AFP, significant accumulations of both SA and AFP were demonstrated in the tumors, SA being about 3-fold higher than that of AFP after normalization to quantity of uptake in liver. In the case of comparatively studied neuroblastoma presenting only neuroblastic components (different from teratocarcinoma containing both neuroectoblastic and neuroblastic elements), the accumulation of radiolabeled SA and AFP showed relationship 1:1. External *in vivo* photo scanning confirmed this relationship of accumulated radiolabeled proteins in both studied tumors; the last observations were useful for differential diagnosis of tumors [4, 27–36].

AFP may be used to advantage in radio tracing experiments, since this isologous protein is not expected to induce hypersensitivity reactions. On the other hand, and contrary to SA, the extremely low serum levels of AFP in adult individuals should minimize effects due to competition with endogenous protein. This makes AFP a good candidate for tumor biomarker by imaging techniques. The diagnosis and therapies of CNS tumors including neuroblastoma are always a subject of discussion [36–39].

### 2.2 IGF-I

Another oncodevelopmental antigen, an insulin like-growth factor, IGF-I [20, 40–43], is present in glioma cells but absent in neuroblastoma cells [18]; neuroblastic cells express IGF-II [27]. These observations permitted to study separately, using IGF-I and IGF-II as the oncoprotein markers, glial and neural tumors [13, 15, 20, 40–42, 44–46].

Comparative studies of AFP, IGF-I, IGF-II presence in neoplastic cells [3, 4, 18] have demonstrated that IGF-I constitutes an essential target for genetic testing. IGF-I, similarly to AFP, is involved in tissue development and differentiation, especially in the development of the nervous system [9, 15, 19, 20, 47–51]. According to

Baserga [43], IGF-I is one of the most important growth factors related to normal and neoplastic differentiation [50, 52–54].

The elements of IGF-I related transduction pathway (IRS/PI3K-PKC/PDK1/AKT-Bcl2/GSK3/GS) [55, 56] were also considered as targets for diagnostic [9, 55, 57–66]. The relationship between IGF-I and IGF binding proteins are being introduced in clinical diagnostics as one of the indicators of precancerous development [67].

IGF-I becomes useful in molecular diagnostic of neonatal CNS malformations and tumors [5, 9, 13, 39, 68, 69]. Diagnosis and treatment should logically be related, at first using IGF-I gene testing for diagnosis [13, 21, 70], and then targeting IGF-I gene through special therapy, such as cancer gene therapy, especially therapy of gliomas [11, 22, 71–73].

### 3. IGF-I and anti-gene immunotherapy

#### 3.1 Protein, translation and transcription levels

To target an oncoprotein directly on protein level, the strategy of antibodies was explored. Cancers treated by use of antibodies was in general not efficient.

The treatment of any cancer, especially hepatocarcinoma, demands a permanent perfusion, *per vena porta*, of anti AFP antibodies. The arrest of perfusion has produced the reappearance of cancer. Similar observation has been made when using antibodies against growth factors.

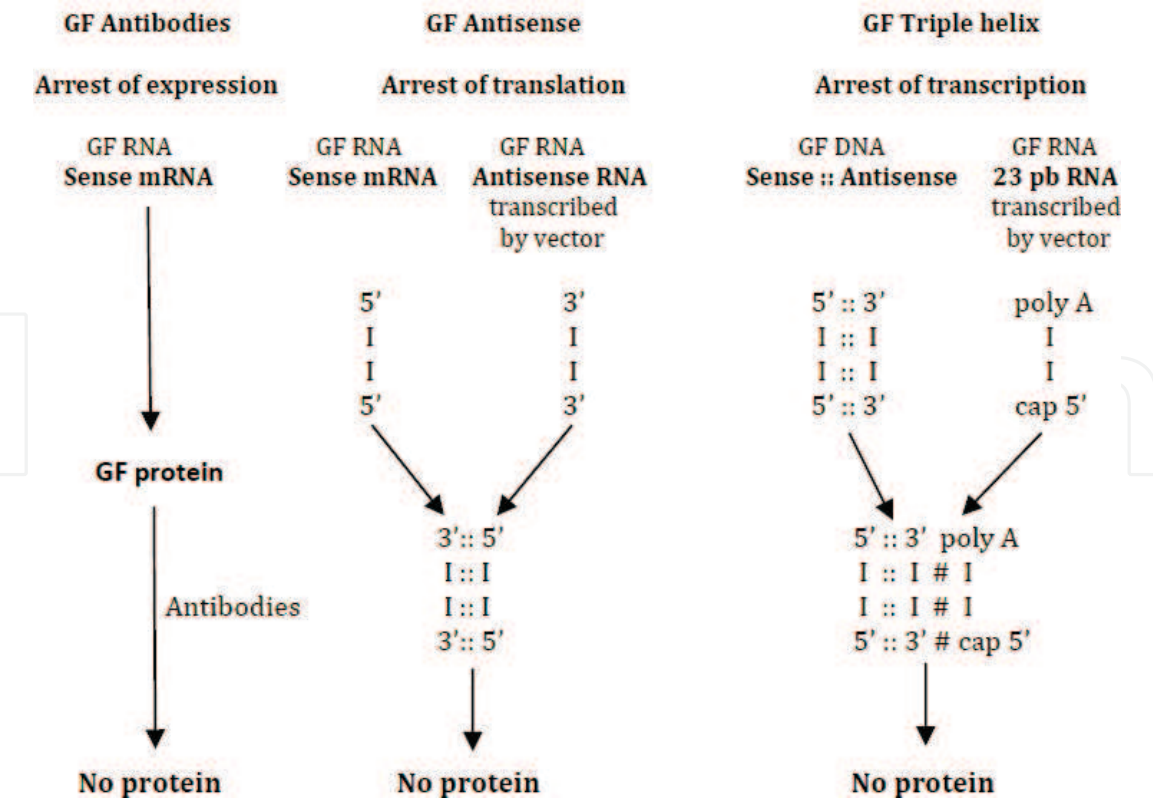
The only possibility was to stop the synthesis of the oncoprotein on the translation or on the transcription level of the concerned gene, and directly in the cancer cells. This hypothesis and our knowledge of chemistry was an epistemological problem, pointed out by Mosquera “how to integrate the knowledge of chemistry with technique” [74].

As to glioma malignant tumor, glioblastoma (the mortality remains close to 100%), new or proposed therapies are based generally either on immune treatment or on immuno-gene strategies [75, 76]. In order to define new therapies, the different techniques for inhibitors [9], and the anti-gene strategy (either antisense, AS, or triple helix, TH, approaches) were investigated [61] (**Figure 1**).

The AS technology [77, 78] has permitted us to establish new and successful gene therapy strategies targeting glioma's growth factors [11, 79] and have now been introduced into clinical trials. Other recently introduced technologies include those of triple helix, TH [80–83], as well as potentially useful siRNA [84, 85] and miRNA (microRNA) [82, 83, 86, 87]. The role of 21–23 mer double-stranded RNA (siRNA) in the silencing of genes is strongly similar to that of the TH DNA mechanism, which also involves 23 mer RNA [81]. Whether or not siRNA technology or miRNA knockdown will supplant the AS and TH oligodeoxynucleotide approaches remains in question at this time [83, 85, 87–90]. AS methodology is currently being standardized to be largely used in clinical trials [18, 89].

As to TH strategy, the oligonucleotides are targeted to double stranded DNA containing polypurine-polypyrimidine sequences that readily form triple helices. The studies of triple helix strategy have shown that an RNA strand containing a 23-nucleotide (nt) oligopurine sequence [80, 86, 87] may be capable of forming triple helix structures with an oligopurine-oligopyrimidine sequence of the IGF-I gene as well in cultured rat C6 glioma as in rat CNS-1 glioma, and in mouse PCC-4 cells [86]. Although we cannot exclude other mechanisms, triple helix formation remains the most plausible possibility for the inhibition of IGF-I gene expression [86]. The arrest of IGF-I synthesis suggests that the RNA strand, which forms the triple helix, has inhibited gene transcription in glioma cells.





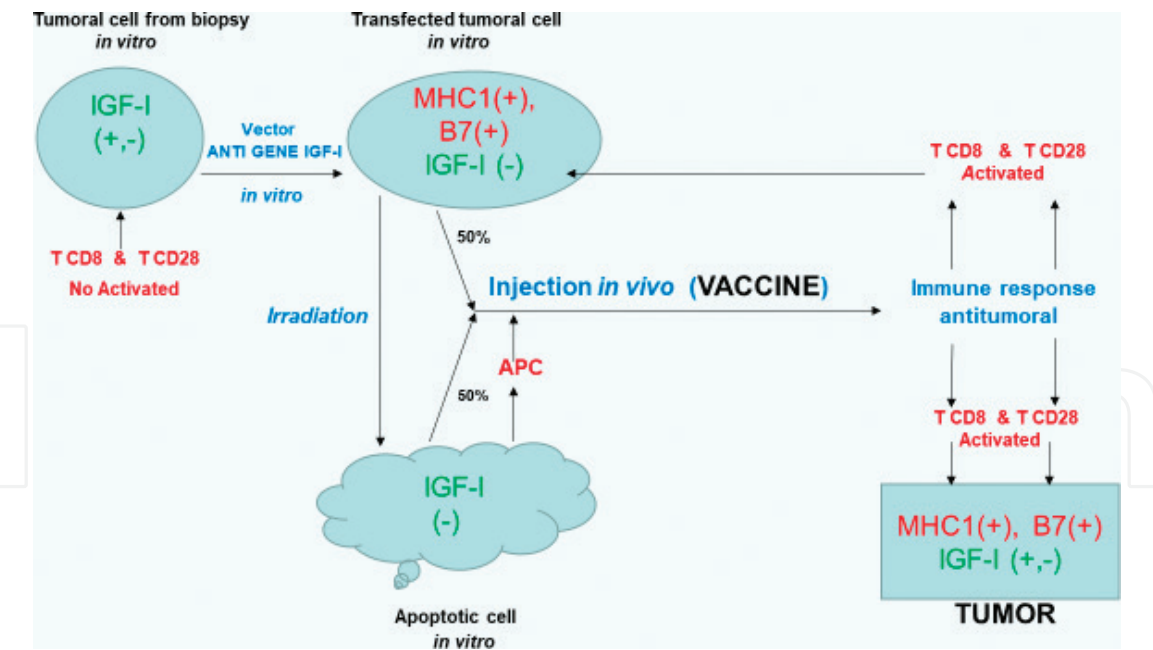
**Figure 1.** Schema of technologies suppressing the expression of oncoproteins, including growth factors, GF: antibodies, antisense and triple helix. In antibodies technology, the GF expression is directly blocked by its ligand—antibody. In antisense technology, the end result is the inhibition of GF mRNA (sense RNA) activity by binding to the antisense RNA. In triple helix technology, the oligopurine third strand (23 bp) forms RNA-DNA triple helix with GF gene.

3.2 Antisense and triple helix experimental studies

In our experimental studies, we have shown that in IGF-I “antisense” and “triple helix” transfected CNS-1 rat glioma cells, PCC-4 mouse embryonal carcinoma cells, and in primary human glioma cells, changes in immunogenic properties and apoptosis occur. The induction of IGF-I triple helix forming structure, similarly to IGF-I antisense approach, was followed by enhanced expression of MHC-I and B-7 [5, 18, 90–95] and loss of *in vivo* tumorigenicity. An extensive lymphocytic CD8 positive infiltration 4–5 days after injection of AS transfected glioma, teratocarcinoma and hepatoma cells into the respective animal bearing tumors was demonstrated [11, 27, 96]. These properties were used for selection of human glioma cells that were used in IGF-I antisense/triple helix immunogene therapy [90] (Figure 2).

In this context, antigenic peptides presented by class I MHC molecules were necessary but, in general, not sufficient to stimulate T cell response. In the absence of B-7 molecule, MHC-peptide complexes could selectively inactivate T-cells [97]. (Although “triple helix” cells as compared to “antisense” cells show slightly higher expression of MHC-I and B7 there are no qualitative immunogenic and apoptotic differences between IGF-I “antisense” and “triple helix” approaches).

The absence of IGF-I synthesis in AS and TH transfected cells, could lead to a higher level of IGF-I receptor and hence to greater tyrosine kinase content [98]. There is a relation between the signal transduction pathway of tyrosine kinase and induction of B-7 molecules: enhancement in B-7 co-stimulation through a cAMP mechanism linked to tyrosine kinase of the CD 28 receptor has been previously reported [99]. Similar signaling through the tyrosine kinase activity of the IGF-I receptor shows that: tyrosine kinase activates IRS-1 (Insulin receptor substrate-1),



**Figure 2.**  
Schema of cancer immunogene therapy. The cells isolated from tumor biopsy are growing in tissue culture. The established cell line is transfected by vector of anti-gene type (antisense and triple helix IGF-I). The transfected cells, and originated apoptotic cells, are injected in proportion 50–50 (“vaccine”) in the cancer patients. The presence of MHC-I and B7 molecules present in transfected tumor cells induce an immune response mediated by T lymphocytes. Moreover, APC cells also participate as shown in the immunogene mechanism. The activated T lymphocytes destroy as well the vaccine (transfected tumor cells) as a tumor.

and then IRS-1 activates PI3K (phosphatidylinositol 3 kinase) [100, 101]. This mechanism could be considered in the cytokine induced B7–1 expression demonstrated in fetal human microglia in culture [102].

In the AS and TH transfected cells, IGF-I-R activated by its ligand plays a very protective role in programmed cell death, and that this protection is even more striking *in vivo* than *in vitro* [103]. Apoptosis could play a specific role in our strategies; the phenotypic modifications due to apoptosis may explain the recognition of the transfected cells by the immune system like tumor-specific immunity mediated by CD8+ T [11, 98]. Apoptotic cells, in the context of MHC-I are recognized by dendritic cells activating lymphocytes T-CD8 [104, 105]. B-7 molecules can be included in this mechanism, because both MHC-I and B-7 molecules are necessary for T cell activation [16, 27, 36, 91, 106–108].

While working on mouse hepatoma cells transfected by the IGF-I triple helix approach, we have observed a decrease of cytokines such as Il-10, which is a strong immunosuppressor [109], and TNF-alpha, which can act as a factor stimulating tumor growth [110, 111]. Moreover, we have found increased levels of TAP 1 and 2 in these cells. The relationship between the immune process, related to the MHC-I or HLA system [112, 113], and the apoptotic process is under study.

### 3.3 Anti-gene clinical therapy

In the clinical trial using anti-gene IGF-I therapy after subcutaneous vaccination, the subject developed peri-tumor necrosis; the tissue section bordering the necrotic tumor tissue showed infiltration of lymphocytes consisting of both CD4 and CD8 T cells [114]. Other results concerning peripheral blood lymphocytes (PBL) of glioblastoma patients treated by IGF-I triple helix immuno-gene therapy show eminent changes in CD8 T cells, especially after second injection. There was also an increase in the percentage of CD8 with characteristics switching from CD8+

CD11b<sup>+</sup> to CD8<sup>+</sup> CD11b<sup>-</sup> phenotype, an alteration that may reflect the enhanced activation of T cytotoxic cells in blood. Additionally, as far as prognosis of glioblastoma patients is concerned, the patients have survived between 18 and 24 months after the first diagnosis. Generally, patients with diagnosed glioblastoma multiforme, undergoing surgeries and radiotherapy, survive up to 14 months [22, 113].

Our phase I human cancer clinical trial based on the anti-gene, antisense approach, is in progress in the U.S.A, Thailand, Poland and China [107, 114, 115] and is in parallel with previously established approaches of glioblastoma treatment established by either Culver [116] or by Baserga [65]. Following the same approach, different antisense strategies to treat glioma tumors have been recently investigated [26, 31, 117, 118], especially in relation to glycogen metabolism [119] in glioma cells. (Malignant astrocytes present a high level of glycogen [55]). The therapy of gliomas using strategies of growth factors inhibition is in permanent progress. The inhibition of the IGF-I receptor using AS technology, and its signaling pathway has opened a door for experimental and clinical research in various tumors [9, 14, 15, 42, 62, 120, 121].

#### **4. Discussion**

The use of anti-gene therapy was also effective in another AS approach, targeted toward the molecule, TGF-beta [79]. TGF-beta2 plays a role in tumor progression by regulating key mechanisms including proliferation, metastasis, and angiogenesis. The approach of AS TGF-beta using an AS oligodeoxynucleotide—compound AP 12009, has given satisfactory results [122–124]. AP-12009 treatment was well tolerated and tumor response has been observed [123]. Two patients experienced long-lasting complete tumor remission [124]. In another clinical AS TGF-beta study, a phase I clinical trial in grade IV astrocytoma (GBM) was performed using autologous tumor cells modified by a AS TGF-beta2 vector [79]. There were indications of humoral and cellular immunity induced by the vaccine [57, 79].

As far as AS TGF-beta therapy of GBM is concerned, the treatment of patients with recurrent or refractory malignant (high-grade) glioma, WHO grade III or IV has been shown to produce results, similar to results obtained with anti IGF-I treatment. The role of peripheral blood mononuclear cells in the immune antitumor response, and prolonged survival in both anti TGF beta and anti IGF-I approaches was comparatively examined. In the case of TGF-beta, it is also important to mention, that although the first successful clinical results were published in 2006–2008, the solid experimental data, using AS technology were obtained in 1994/95 [79], which means, that long periods of research on the mechanism of AS TGF-beta and AS IGF I are required before achieving significant clinical results, confirming the usefulness of gene therapy in cancer treatment [90, 122, 123, 125–130].

In AS IGF-I or AS TGF-beta approaches, immune antitumor response, mediated by TCD8 and APC cells, was signaled as a principal mechanism of AS technology inhibiting growth factors and their signaling pathway [12, 79]. These cells being involved in HS (heat shock) protein mechanism, the inhibition of HS was introduced in clinical trials as a new direction for cancer therapy [131].

More recent clinical successful strategies of gliomas treatment, generally as a combination therapy using different types of inhibitors (i.e., imatinib, gefitinib) including antibodies (i.e., avastin) targeting growth factors and their receptors [132–136], are now focusing on anti-gene strategy, especially on antisense or triple helix technologies used alone or combined also with pharmacological treatment. As far as antisense and triple helix strategies are compared, the triple helix blockade of

growth factor has given better results *in vitro* and *in vivo*. But combining both strategies, the final result is most effective—stopping the expression of the oncoproteins on translation (AS) and transcription (TH) levels, respectively.

## 5. Conclusion

Among the new strategies in the efforts to successfully treat GBM, the use of AS approach targeting IGF-I, TGF-beta or VEGF, their receptors and their downstream transduction signaling elements [9, 137], appears to offer a promising solution. The final result of the signal transduction pathway element inhibition is an immune response mediated *in vivo* by lymphocytes T CD8 and APC cells. Using cancer immunogene therapy of anti-gene anti IGF-I approach, the median survival of treated glioblastoma patients has reached 22–24 months. But the near future in treating this group of disorders belongs to a combination of treatment: classical surgery, radiotherapy with immunotherapy, pharmacologic therapy, growth factor inhibitors, and the use of the antisense/triple helix gene blockade approach targeting signal transduction pathway elements of cancer processes [16, 22, 23, 36, 66, 90, 125, 138–147].

## Conflict of interest

No conflict of interest.

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