We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

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⁺ to CD8⁺ CD11b⁻ 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.

Author details

Jerzy Trojan^{1,2,3,4*}

1 Laboratories Legrand, Bogota, Colombia

2 ICGT–International Cancer Gene Therapy, Bogota, Colombia

3 ICGT–International Cancer Gene Therapy, Paris, France

4 INSERM, Paris, France

*Address all correspondence to: genetherapy@hotmail.fr

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Abelev GJ. Alpha-fetoprotein in ontogenesis and its association with malignant tumors. *Advances in Cancer Research*. 1971;**14**:295-299
- [2] Trojan J, Uriel J, Deugnier MA, Gaillard J. Immunocytochemical quantitative study of alphafetoprotein in normal and neoplastic neural development. *Developmental Neuroscience*. 1984;**6**:251-259
- [3] Hajeri-Germond M, Naval J, Trojan J, et al. The uptake of alphafetoprotein by C-1300 mouse neuroblastoma cells. *British Journal of Cancer*. 1985;**51**:791-797
- [4] Trojan J, Naval X, Johnson T, et al. Expression of serum albumin and of alphafetoprotein in murine normal and neoplastic primitive embryonic structures of teratocarcinoma. *Molecular Reproduction and Development*. 1995;**42**(4):369-378
- [5] Harding BN, Golden JA. *Developmental Neuropathology*. Basel, Switzerland: International Society of Neuropathology; 2004
- [6] Calaminus G, Bamberg M, Harms D, et al. AFP/ β -HCG secreting CNS germ cell tumors: Long-term outcome with respect to initial symptoms and primary tumor resection. Results of the Cooperative Trial MAKEI 89. *Neuropediatrics*. 2005;**36**(2):71-77
- [7] Kim A, Ji L, Balmaceda C, et al. The prognostic value of tumor markers in newly diagnosed patients with primary central nervous system germ cell tumors. *Pediatric Blood & Cancer*. 2008;**51**(6):768-773
- [8] Kawaguchi T, Kumabe T, Kanamori M, et al. Logarithmic decrease of serum alpha-fetoprotein or human chorionic gonadotropin in response to chemotherapy can distinguish a subgroup with better prognosis among highly malignant intracranial non-germinomatous germ cell tumors. *Journal of Neurooncology*. 2011;**104**(3):779-787
- [9] Trojan J, Cloix J-F, Ardourel M, et al. IGF-I biology and targeting in malignant glioma. *Neuroscience*. 2007;**145**(3):795-811
- [10] Kiess W, Lee L, Graham DE, et al. Rat C6 glial cells synthesize insulin-like growth factor I (IGF-I) and express IGF-I re-ceptors and IGF-II/mannose 6-phosphate re-ceptors. *Endocrinology*. 1989;**124**:1727-1736
- [11] Trojan J, Johnson T, Rudin S, et al. Treatment and prevention of rat glioblastoma by immugenic C6 cells expressing antisense insulin-like growth factor I RNA. *Science*. 1993;**259**:94-97
- [12] Ly A, Duc HT, Kalamarides M, et al. Human glioma cells transformed by IGF-I tri-ple-helix technology show immune and apoptotic characteristics determining cell selection for gene therapy of glioblastoma. *Journal of Clinical Pathology (Molecular Pathology)*. 2001;**54**:230-239
- [13] Zumkeller W. IGFs and IGF-binding proteins as diagnostic markers and biological modulators in brain tumors. *Expert Review of Molecular Diagnostics*. 2002;**2**:473-477
- [14] Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. *Nature Reviews. Cancer*. 2004;**4**:505-518. DOI: 10.1038/nrc1387
- [15] Baserga R. The insulin-like growth factor-I receptor as a target for cancer therapy. *Expert Opinion on Therapeutic Targets*. 2005;**9**:753-768

- [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(3):e58428. DOI: 10.1371/0058428
- [17] Trojan A, Jay LM, Briceño I, et al. IGF-I, IGF-I gene and diagnostic. In: Trojan J, editor. *Cancer Immunogene Therapy. Anti-Gene Anti IGF-I Approach. Case of Glioblastoma*. Saarbrücken, Germany: Lambert Academic Publishers; 2017. pp. 7-28. ISBN-13: 978-3-330-06345-7; ISBN-10: 3330063459
- [18] Trojan J, Blossey BK, Johnson T, et al. Loss of tumorigenicity of rat glioblastoma directed by episome-based antisense cDNA transcription of insulin-like growth factor I. *Proceedings of the National Academy of Sciences of the United States of America*. 1992;89(11):4874-4878
- [19] Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis. *Endocrine Reviews*. 2011;22(1):53-74. DOI: 10.1210/edrv.22.1.0419
- [20] Froesch CS, Schwander J, Zapf J. Actions of insulin-like growth factors. *Annual Review of Physiology*. 1985;47:443-467
- [21] Johnson TR, Trojan J, Rudin SD, et al. Effects on actinomycin D and cycloheximide on transcript levels of IGF- I, actin and albumin in hepatocyte primary cultures treated with growth hormone and insulin. *Molecular Reproduction and Development*. 1991;30:95-99
- [22] Trojan A, Jay LM, Kasprzak H, et al. Immunotherapy of malignant tumors using antisense anti-IGF-I approach: Case of glioblastoma. *Journal of Cancer Therapy*. 2014;5:685-705
- [23] Trojan J. Anti – Gene anti IGF-I technology applied for cancer immunotherapy. *World Journal of Research Review*. 2016;1(3):67-75
- [24] Trojan J, Uriel J. Immunocytochemical signaling of alpha fetoprotein (AFP) and serum-albumin (Alb) in ecto-, mesa- and endodermal tissue derivatives of the developing rat. *Oncodevelopmental Biology and Medicine*. 1982;3:12-22
- [25] Benno RH, Williams TH. Evidence for intracellular localization of AFP in the developing rat brain. *Brain Research*. 1978;142:182-186
- [26] Trojan J, Uriel J. Localisation intracellulaire of l'AFP et de la serum albumine dans le fœtal nerveux central du rat au cours du développement fœtal et postnatal. *Comptes Rendus de l'Académie des Sciences Paris*. 1979;289:1157-1160
- [27] Trojan J, Johnson TR, Rudin SD, et al. Gene therapy of murine teratocarcinoma: Separate functions for insulin-like growth factors I and II in immunogenicity and differentiation. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;91:6088-6092
- [28] Schubert D, Humphreys S, Baroni C, Cohn M. In vitro differentiation of a mouse neuroblastoma. *Biochemistry*. 1969;64:316-323
- [29] Bernal S, Thompson RA, Gilbert F, Baylin S. In vitro and in vivo growth characteristics of two different cell populations in an established line of human neuroblastoma. *Cancer Research*. 1983;43:125-164
- [30] Uriel J, Faivre-Baumann A, Trojan J, et al. Immunocytochemical demonstration of alphafoetoprotein uptake by primary cultures of foetal

hemisphere cells from mouse brain. *Neuroscience Letters*. 1981;**27**:171-175

[31] Uriel J, Poupon MF, Geuskens M. Alpha-fetoprotein uptake by cloned cell lines derived from a nickel-induced rat rhabdomyosarcoma. *British Journal of Cancer*. 1983;**48**:261-269

[32] Uriel J, Villacampa MJ, Moro R, et al. Uptake of radiolabeled alpha-fetoprotein by mouse mammary carcinomas and usefulness in tumor scintigraphy. *Cancer Research*. 1984;**44**:5314-5319

[33] Gaillard J, Caillaud JM, Maunoury R, et al. Expression du neuroectoblaste dans le teratocarcinome et le teratome de la souris. *Bulletin de l'Institut Pasteur*. 1984;**82**:335-385

[34] Trojan J, Uriel J, Gaillard JA. Localisation de alpha-fetoprotéine dans les dérivés neuroépithéliaux des teratocarcinomes de la souris. *Annales de Pathologie*. 1983;**3**:137-145

[35] Hajeri-Germond M, Trojan J, Uriel J, et al. In vitro uptake of exogenous alpha-fetoprotein by chicken dorsal root ganglia. *Developmental Neuroscience*. 1984;**6**:111-117

[36] Castillo T, Trojan A, Noguera MC, et al. Epistemologic experience in elaboration of molecular biology technology for immunogene therapy (in Spanish). *Revista Científica*. 2016;**2**:25. DOI: 10.14483/udistrital.jour.RC.2016.25.a6

[37] 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 Research*. 2010;401-427. DOI: 10.1155/2010/427401

[38] Lichter T. Evolution of the Molecular Biology of Brain Tumors and the Therapeutic Implications. Vienna/Rijeka: InTech; 2013

[39] Love S, Arie Perry A, Ironside J, Budka H. *Greenfield's Neuropathology*. 9th ed. New York: CRC Press; 2015

[40] Daughaday WH, Hall K, Raben MS, et al. Somatomedin: Proposed designation for sulphation factor. *Nature*. 1972;**235**:107

[41] Han VKM, Hill DJ. In: Shofield PN, editor. *The Insulin-Like Growth Factors: Structure and Biological Functions*. Oxford, England: Oxford University Press; 1992. pp. 178-219

[42] Baserga R, Sell C, Porcu P, Rubini M. The role of the IGF-I receptor in the growth and transformation of mammalian cells. *Cell Proliferation*. 1994;**27**:63-71

[43] Baserga R. Oncogenes and the strategy of growth factors. *Cell*. 1994;**79**:927-930

[44] Sturm MA, Conover CA, Pham H, et al. Insulin like growth factor receptors and binding proteins in rat neuroblastoma. *Endocrinology*. 1989;**124**:388-396

[45] Werther GA, Abate M, Hogg A, et al. Localisation of Insulin like growth factor mRNA in rat brain by in situ hybridization-relation to IGF-I receptor. *Molecular Endocrinology*. 1990;**4**:773-778

[46] Antoniades HN, Galanopoulos T, Neville-Golden J, et al. Expression of insulin like growth factor I and II and their receptor mRNAs in primary human astrocytomas and meningiomas: In vivo studies using in situ hybridization and immunocytochemistry. *International Journal of Cancer*. 1992;**50**:215-222

[47] Trojan J, Uriel J, Deugnier MA, et al. Immunocytochemical quantitative study of alpha-fetoprotein in normal and neoplastic neural development. *Developmental Neuroscience*.

1984;**6**(4-5):251-259. DOI:
 10.1159/000112352

[48] Ostos H, Astaiza G, Garcia F, et al. Decreased incidence of defects of neural tube closure at the University Hospital of Neiva: Possible effect promotion of folic acid (in Spanish). *Biomédica*. 2000;**20**(1):18-24. DOI: 10.7705/ignaling.v20i1.1043

[49] Le Roith D. The insulin-like growth factor system. *Experimental Diabetes Research*. 2003;**4**(4):205-212. DOI: 10.1155/EDR.2003.205

[50] Adhami VM, Afaq F, Mukhtar H. Insulin-like growth factor-I axis as a pathway for cancer chemoprevention. *Clinical Cancer Research*. 2006;**12**(19):5611-5614. DOI: 10.1158/1078-0432.CCR-06-1564

[51] Chen H, Mester T, Raychaudhuri N, et al. Teprotumumab, an IGF-1R blocking monoclonal antibody inhibits TSH and IGF-1 action in fibrocytes. *Journal of Clinical Endocrinology and Metabolism*. 2014;**99**(9):E1635-E1640. DOI: 10.1210/jc.2014-1580

[52] Kooijman R. Regulation of apoptosis by insulin-like growth factor (IGF)-I. *Cytokine & Growth Factor Reviews*. 2006;**17**(4):305-323. DOI: 10.1016/j.cytogfr.2006.02.002

[53] Bueno S, Santandr R, Alvarez A, et al. Brain stem cells and IGF-I: Implications in development, regeneration and cancer therapeutics. *Integrative Molecular Medicine*. 2018;**5**(1). DOI: 10.15761/IMM.1000319

[54] Kurmasheva RT, Houghton PJ. IGF-I mediated survival pathways in normal and malignant cells. *Biochimica et Biophysica Acta*. 2006;**1766**(1):1-22. DOI: 10.1016/j.bbcan.2006.05.003

[55] Beckner ME, Gobbel GT, Abounader R, et al. Glycolytic glioma cells with active glycogen synthase are

sensitive to PTEN and inhibitors of PI3K and gluconeogenesis. *Laboratory Investigation*. 2005;**85**(12):1457-1470. DOI: 10.1038/labinvest.3700355

[56] Vignot S, Faivre S, Aguirre D, et al. mTOR-targeted therapy of cancer with rapamycin derivatives. *Annals of Oncology*. 2005;**16**(4):525-537

[57] Schlingensiepen KH, Jaschinski F, Lang SA, et al. Transforming growth factor-beta 2 gene silencing with trabedersen (AP 12009) in pancreatic cancer. *Cancer Science*. 2011;**102**(6):1193-1200. DOI: 10.1111/j.1349-7006.2011.01917.x

[58] Trojan J, Anthony DD. Antisense strategies in therapy of gliomas. *Current Signal Transduction Therapy*. 2011;**6**(3):411-423. DOI: 10.2174/157436211797483895

[59] Patel S, Doble B, Woodgett JR. Glycogen synthase kinase-3 in insulin and Wnt signaling: A double-edged sword? *Biochemical Society - Translation UK*. 2004;**32**(5):803-808. DOI: 10.1042/BST0320803

[60] Jiang R, Mircean C, Shmulevich I, et al. Pathway alterations during glioma progression revealed by reverse phase protein lysate arrays. *Proteomics*. 2006;**6**(10):2964-2971. DOI: 10.1002/pmic.200500555

[61] Hutterer M, Gunsilius E, Stockhammer G. Molecular therapies for malignant glioma. *Wien Medicinal Wochenschrift*. 2006;**156**(11):351-363. DOI: 10.1007/s10354-006-0308-3

[62] Sachdev D, Yee D. Disrupting insulin-like growth factor signaling as a potential cancer therapy. *Molecular Cancer Therapy*. 2007;**6**(1):1-12. DOI: 10.1158/1535-7163.MCT-06-0080

[63] Ardourel M, Blin M, Moret JL, et al. A new putative target for antisense gene therapy of glioma: Glycogen synthetase.

- Cancer Biology and Therapy. 2007;**6**(5):719-723. DOI: 10.4161/cbt.6.5.4232
- [64] 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. Laboratory Investigation. 2010;**90**:144-155. DOI: 10.1038/labinvest.2009.126
- [65] Premkumar DR, Arnold B, Jane EP, et al. Synergistic interaction between 17-AAG and phosphatidylinositol 3-kinase inhibition in human malignant glioma cells. Molecular Carcinogenesis. 2006;**45**(1):47-59. DOI: 10.1002/mc.20152
- [66] Sanson M, Laigle-Donadey F, Benouaich-Amiel A. Molecular changes in brain tumours: Prognostic and therapeutic impact. Current Opinion in Oncology. 2006;**18**(6):623-630. DOI: 10.1097/01.cco.0000245322.11787.72
- [67] Trojan J, Briceno I. IGF-I antisense and triple-helix gene therapy of glioblastoma. In: Pantar A, editor. Evolution of the Molecular Biology of Brain Tumors and the Therapeutic Implications. Vienna/Rijeka: InTech; 2013. pp. 149-166
- [68] Esiri M, Perl D. Oppenheimer's Diagnostic Neuropathology. 3rd ed. FL: CRC Press; 2006
- [69] Glick RP, Lichtor T, Unterman TG. Insulin-like growth factors in central nervous system tumors. Journal of Neuro-Oncology. 1997;**35**(3):315-325
- [70] Obrepalska-Steplowska A, Kedzia A, Trojan J, et al. Analysis of coding and promoter sequences of the IGF-I gene in children with growth disorders presenting with normal level of growth hormone. Journal of Pediatric Endocrinology and Metabolism. 2003;**16**(9):1267-1275
- [71] Hu B, Niu X, Cheng L, et al. Discovering cancer biomarkers from clinical samples by protein microarrays. Proteomics: Clinical Applications. 2015;**9**(1-2):98-110. DOI: 10.1002/prca.201400094
- [72] 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 Medizinische Wochenschrift. 2014;**164**(17-18):343-352. DOI: 10.1007/sl 0354-014-0299-4
- [73] Gu F, Schumacher FR, Canzian F, et al. Eighteen insulin-like growth factor pathway genes, circulating levels of IGF-I and its binding protein, and risk of prostate and breast cancer. Cancer Epidemiology, Biomarkers & Prevention. 2010;**19**(11):2877-2887. DOI: 10.1158/1055-9965.EPI-10-0507
- [74] Mosquera CJ, Castelblanco OL. Didactic questions and epistemology (in Spanish). Revista Gondola. 2015;**10**(1). DOI: 10.14483/udistrital.jour.GDLA.2015.1.a00
- [75] Stupp R, Hegi ME, van den Bent MJ, et al. Changing paradigms—An update on the multidisciplinary management of malignant glioma. The Oncologist. 2006;**11**:165-180
- [76] Kjaergaard J, Wang L, Kuriyama H, Shu S, Plautz GE. Active immunotherapy for advanced intracranial murine tumors by using dendritic cell-tumor cell fusion vaccines. Journal of Neurosurgery. 2005;**103**:156-164
- [77] Rubenstein JL, Nicolas JF, Jacob F. Nonsense RNA: A tool for specifically inhibiting the expression of a gene in vivo. Comptes Rendus de l'Académie des Sciences, Paris III. 1984;**299**:271-274
- [78] Weintraub H, Izant J, Harland R. Antisense RNA as a molecular tool

for genetic analysis. *Trends in Genetics*. 1985;1(1):23-25

[79] Fakhrai H, Dorigo O, Shawler DL, et al. Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;93(7):2909-2914

[80] Dervan P. Reagents for the sitespecific cleavage of megabase DNA. *Nature*. 1992;359:87-88

[81] Hélène C. Control of oncogene expression by antisense nucleic acid. *European Journal of Cancer*. 1994;30A:1721-1726

[82] Berezikov E, Thuemmler F, van Laake LW, et al. Diversity of microRNAs in human and chimpanzee brain. *Nature Genetics*. 2006;38(12):1375-1377

[83] 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 Research*. 2007;67(19):8994-9000

[84] Boado RJ. RNA interference and nonviral targeted gene therapy of experimental brain cancer. *NeuroRx*. 2005;2(1):139-150

[85] Pai SI, Lin YY, Macaes B, et al. Prospects of RNA interference therapy for cancer. *Gene Therapy*. 2006;13(6):464-477

[86] 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 Therapy*. 1997;4:105-112

[87] Rininsland F, Johnson T, Chernicky C, et al. Suppression of insulin-like

growth factor type-I receptor by a triple-helix strategy inhibits IGF-I transcription and tumorigenic potential of rat C6 glioblastoma cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;94:5854-5859

[88] Dias N, Stein CA. Basic concepts and antisense oligonucleotides mechanisms. *Molecular Cancer Therapeutics*. 2002;1:347-355

[89] Wells DJ. Therapeutic restoration of dystrophin expression in Duchenne muscular dystrophy. *Journal of Muscle Research and Cell Motility*. 2006;27(5-7):387-398

[90] Trojan J, Pan YX, Wei MX, et al. Methodology for anti-gene anti-IGF-I therapy of malignant tumours. *Chemotherapy Research and Practice*. 2011/2012. DOI: 10.1155/2012/721873

[91] Townsend SE, Allison JA. Tumor rejection after direct costimulation of CD8+ T cell transfected melanoma cells. *Science* 1993;259: 368-370

[92] Guo Y, Wu M, Chen H, et al. Effective tumor vaccine generated by fusion of hepatoma cells with lymphocytes B cells. *Science*. 1994;263:518-520

[93] Trojan J. *Cancer Immunogene Therapy. Anti-Gene Anti IGF-I Approach. Case of Glioblastoma*. Germany: Lambert Academic Publishers; 2017. 140p. ISBN-13: 978-3-330-06345-7; ISBN-10: 3330063459

[94] Linsley PS, Clark EA, Ledbetter JA. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/13. *Proceedings of the National Academy of Sciences of the United States of America*. 1990;87:5031-5035

[95] Freeman GB, Gray GS, Gimmi CD, et al. *Structure*

- [96] Upegui-Gonzalez LC, Ly A, Sierzega M, et al. IGF-I triple helix strategy in hepatoma treatment. *Hepato-Gastroenterology*. 2001;**48**:660-666
- [97] Steiman RM, Turkey S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. *Journal of Experimental Medicine*. 2000;**191**:411-416
- [98] Lafarge-Frayssinet C, Sarasin A, Duc HT, et al. Gene therapy for hepatocarcinoma: Antisense IGF-I transfer into a rat hepatoma cell line inhibits tumorigenesis into syngeneic animal. *Cancer Gene Therapy*. 1997;**4**:276-285
- [99] Schwartz RH. Costimulation of T lymphocytes: The role of CD28, CTLA-4 and B7/BB1 in interleukin-2 production and immunotherapy. *Cell*. 1992;**71**:1065-1068
- [100] D'Ambrosio C, Ferber A, Resnicoff M, et al. A soluble insulin-like growth factor receptor that induces apoptosis of tumor cells in vivo and inhibits tumorigenesis. *Cancer Research*. 1996;**56**:4013-4020
- [101] Dudek H, Datta SR, Franke TS, et al. Regulation of neuronal survival by the serine-threonine protein kinase. *Akta Science*. 1997;**275**:661-665
- [102] Satoh J, Lee YB, Kim SU. T-cell costimulatory molecules B7-1 (CD80) and B7-2 (CD86) are expressed in human microglia but not in astrocytes in culture. *Brain Research*. 1995;**704**:95-96
- [103] Resnicoff M, Abraham D, Yutanawiboonchai W, et al. The insulin-like growth factor I receptor protects tumor cells from apoptosis in vitro. *Cancer Research*. 1995;**55**:2463-2469
- [104] Sotomayor E, Fu Y, Lopez-Cepero M, et al. Role of tumor derived cytokines on the immune system of mice bearing a mammary adenocarcinoma. *Journal of Immunology*. 1991;**147**:2816-2823
- [105] Matthew L, Saiter B, Bhardwag N. Dendritic cells acquire antigen from apoptotic cells and induce class I restricted CTL. *Nature*. 1998;**392**:86-89
- [106] Chen L, Ashe S, Brady WA, et al. Costimulation of anti-tumor immunity by the B7 counter receptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell*. 1992;**71**:1093-1102
- [107] Anthony D, Pan Y, Wu S, et al. Ex vivo and in vivo IGF-I antisense RNA strategies for treatment of cancer in humans. *Advances in Experimental Medicine and Biology*. 1998;**451**:27-34
- [108] 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. *Biomedicine & Pharmacotherapy*. 2015;**70**:53-57. DOI: 10.1016/j.biopha.2015.01.002
- [109] Gerard CM, Bruyns C, Delvaux A, et al. Loss of tumorigenicity and increased immunogenicity induced by interleukin-10 gene transfer in B16 melanoma cells. *Human Gene Therapy*. 1996;**7**(1):23-31
- [110] Buck C, Digel W, Schoniger W, et al. Tumor necrosis factor alpha but not lymphotoxin, stimulates growth of tumor cells in hairy cell leukemia. *Leukemia*. 1990;**4**:431-439
- [111] Aggarwal B, Schwarz L, Hogan M, et al. Triple helixforming oligodeoxiribonucleotides targeted to the human tumor necrosis factor (TNF) gene inhibit TNF production and block the TNF dependent growth of human glioblastoma tumor cells. *Cancer Research*. 1996;**56**:5156-5164

- [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. *Proceedings of the National Academy of Sciences of the United States of America*. 1992;**89**(8):3488-3492
- [113] Trojan LA, Ly A, Kopinski P, et al. Antisense and triple helix anti IGF-I tumour vaccines: Gene therapy of gliomas. *International Journal of Cancer Prevention*. 2007;**2**(4):227-243
- [114] Wongkajornslip A, Ouyprasertkul M, Sangruchi T, et al. The analysis of peri-tumor necrosis following the subcutaneous implantation of autologous tumor cells transfected with an episome transcribing an antisense insulin-like growth factor I RNA in a glioblastoma multiforme subject. *Journal of the Medical Association of Thailand*. 2001;**m4**(3):740-747
- [115] Trojan LA, Kopinski P, Mazurek A, et al. IGF I triple helix gene therapy of rat and human gliomas. *Annales Academiae Medicae Bialostocensis*. 2003;**48**:18-27
- [116] Culver KW, Rarn Z, Wallbridge S. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. *Science*. 1992;**256**:1550-1552
- [117] Kopinski P, Ly A, Trojan J. Chapter 44: Antisense in oncology. In: Khayat D, Ly A, editors. *About Cancer in Africa*. Paris, France: Springer; 2005/2006
- [118] Grossman SA, Alavi JB, Supko JG, et al. Efficacy and toxicity of the antisense oligonucleotide aprinocarsen directed against protein kinase C- α delivered as a 21-day continuous intravenous infusion in patients with recurrent high-grade astrocytomas. *Neuro-Oncology*. 2005;**7**(1):32-40
- [119] Bernard-Helary K, Ardourel M, Magistrett P, et al. Stable transfection of cDNAs targeting specific steps of glycogen metabolism supports the existence of active gluconeogenesis in mouse cultured astrocytes. *Glia*. 2002;**37**(4):379-382
- [120] Andrews DW, Resnicoff M, Flanders A, et al. Results of a pilot study involving the use of an antisense oligodeoxynucleotide directed against the insulin-like growth factor type I receptor in malignant astrocytomas. *Journal of Clinical Oncology*. 2001;**19**:2189-2200
- [121] Samani AA, Fallavollita L, Jaalouk DE, et al. Inhibition of carcinoma cell growth and metastasis by a vesicular stomatitis virus G-pseudotyped retrovector expressing type 1 insulin-like growth factor receptor antisense. *Human Gene Therapy*. 2001;**12**:1969-1977
- [122] Schlingensiepen R, Goldbrunner M, Szyrach MNI, et al. Intracerebral and intrathecal infusion of the TGF- β 2-specific antisense phosphorothioate oligonucleotide AP 12009 in rabbits and primates: Toxicology and safety. *Oligonucleotides*. 2005;**15**(2):94-104
- [123] Schlingensiepen KH, Schlingensiepen R, Steinbrecher A, et al. Targeted tumor therapy with the TGF- β 2 antisense compound AP 12009. *Cytokine & Growth Factor Reviews*. 2006;**17**:129-139
- [124] Hau P, Jachimczak P, Schlingensiepen R, et al. Inhibition of TGF- β 2 with AP 12009 in recurrent malignant gliomas: From preclinical to phase I/II studies. *Oligonucleotides*. 2007;**17**(2):201-212
- [125] Schlingensiepen KH, Fischer-Blass B, Schmaus S, et al. Antisense therapeutics for tumor treatment: the TGF- β 2 inhibitor AP 12009 in

clinical development against malignant tumors. *Recent Results in Cancer Research*. 2008;**177**:137-150

[126] Anthony DD. Ex vivo and in vivo IGF-1 antisense RNA strategies for treatment of cancers in humans [Abstract]. *Cancer Gene Therapy*. 1997;**2**(6):S322

[127] 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 [abstract]. *Acta Chirurgica Belgica*. 2003;**5**(103):S2-S3

[128] Schlingensiepen R, Goldbrunner M, Bischof A, et al. Antisense-TGF-beta-2 oligonucleotide AP 12009: Results of safety pharmacology and toxicity studies [abstract]. *Journal of Cancer Research and Clinical Oncology*. 2002;**128**(1):S134

[129] Sierzega M, Jarocki P, Trojan J, et al. Gene immunotherapy of pancreatic cancer using IGF-I antisense approach; preliminary results [Abstract]. *Journal of Hepato-Biliary-Pancreatic Surgery*. 2002;**9**(1):S226

[130] Trojan J, Kopinski P, Drewa T, et al. Immunogenotherapy of prostate cancer. *Urologia Polska*. 2003;**56**(2):7-11

[131] Messaoudi S. Recent advances in Hsp90 inhibitors as antitumour agents. *Anti-Cancer Agents in Medicinal Chemistry*. 2008;**8**:761-782

[132] Goudar RK, Shi Q, Hjelmeland MD, et al. Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. *Molecular Cancer Therapy*. 2005;**4**:101-112

[133] Lamszus K, Brockman MA, Eckerich C, et al. Inhibition of

glioblastoma angiogenesis and invasion by combined treatments directed against vascular endothelial growth factor receptor-2, epidermal growth factor receptor, and vascular endothelial-cadherin. *Clinical Cancer Research*. 2005;**11**:4934-4940

[134] Reardon DA, Quinn JA, Vredenburgh JJ, et al. Phase 1 trial of gefitinib plus sirolimus in adults with recurrent malignant glioma. *Clinical Cancer Research*. 2006;**12**:860-868

[135] Halatsch ME, Schmidt U, Behnke-Mursch L, Untenberg A, Wirtz CR. Epidermal growth factor inhibition for the treatment of glioblastoma multiforme and other malignant brain tumours. *Cancer Treatment Reviews*. 2006;**32**:74-89

[136] Wen PY, Yung WK, Lamborn KR, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: Norty American Brain Tumour Consortium Study 99-08. *Clinical Cancer Research*. 2006;**12**:4899-4907

[137] 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

[138] Trojan LA, Ly A, Upegui-Gonzalez LC, et al. Antisense anti IGF-I therapy of primary hepatic cancer. *Journal Africain du Cancer*. 2009;**1**:1-10

[139] Biroccio A, Leonetti C, Zupi G. The future of antisense therapy: Combination with anticancer treatment. *Oncogene*. 2003;**22**:6579-6588

[140] Meng Y, Carpentier AF, Chen L, et al. Successful combination of local CpG-ODN and radiotherapy in malignant glioma. *International Journal of Cancer*. 2005;**116**:992-997

[141] Gonzalez J, Gilbert MR. Treatment of astrocytomas. *Current Opinion in Neurology*. 2005;**18**:632-638

[142] Jane EP, Premkumar DR, Pollack IF. Coadministration of sorafenib with rottlerin potently inhibits cell proliferation and migration in human malignant glioma cells. *Journal of Pharmacology and Experimental Therapy*. 2006;**319**:1070-1080

[143] Vega EA, Graner MW, Sampson JH. Combating immunosuppression in glioma. *Future Oncology*. 2008;**4**(3):433-442

[144] Trojan J. *Brain - From Development to Neoplasia and Gene Therapy Solution*. Germany: Lambert Academic Publishers; 2018. 150p. ISBN: 978-620-2-08024-8

[145] Dietrich PY, Dutoit V, Tran Thang NN, et al. T cell immunotherapy for malignant glioma: Toward a combined approach. *Current Opinion in Oncology*. 2010;**22**(6):604-610

[146] Wikipedia—Gene Therapy, History 1990s–2010s

[147] Trojan A, Aristizabal B, Jay LM, et al. Testing of IGF-I biomarker in an ethical context. *Advances in Modern Oncology Research*. 2016;**2**(4). DOI: 10.18282/amor.v2:i4.58