# The role of receptor tyrosine kinases in Brain Tumour Diagnosis and Therapy (RTKBT)

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## Scientific Report IV (1st of January to 5th of December, 2014)

**Objectives**

1. Collection of tumors for Brain Tumors Bank maintaining optimum safety of the biological matherial.
2. Determination of the cytotoxic effect of combined cytostatic treatment (with various classes of tyrosine kinase inhibitors) on tumor cell lines.

## Results

Tumors of central nervous system (CNS) are a heterogeneous group of neoplasms with different backgrounds, prognosis and behavior. Depending on the original cell type, glioblastomas and astrocytomas are neoplasms that arise from cells called astrocytes. Glioblastoma and astrocytomas are more resistant to chemotherapy than other types of brain tumors, such as medulloblastomas, oligodendrogliomas, primary brain lymphoma and germ cell tumors. Two types of chemotherapy are generally used in brain cancer: temozolomide (TMZ) and the combination of procarbazine, lomustine and vincristine (PCV). PCV therapy has been used for more than 30 years to treat brain tumors, while TMZ therapy is a new therapeutic approach used both for tumor treatment of high-grade and low-grade astrocytomas, largely replacing the PCV treatment because of the oral administration and minor side effects. Doxorubicin (DOXO) has also been reported as one of the most effective antiproliferative agents against malignant glioma in vitro. However, a poor penetration of the blood-brain barrier makes it difficult as treatment.

A method used to administer the cytostatic DOXO was encapsulation in nanoparticles, for better cross the blood-brain barrier. DOXO administration with certain carriers was also used to increase access of DOXO through blood-brain barrier. More bioactive molecules isolated from plants have been used as anti-cancer agents for leukemia, lymphoma and solid tumors. Most anticancer drugs approved worldwide rely on natural products or their derivatives. For example,

topoisomerase I inhibitors, Irinotecan and Topotecan anticancer drugs are derivatives of Camptothecin, a quinoline-based alkaloid extracted from the shrub Asian Combretum caffrum. A number of other therapy used in the treatment of neoplastic diseases are extracted from plants, for example, Combretastatin, was isolated from Combretum caffrum (South Africa), paclitaxel was isolated from Taxus brevifolia. Cyclopamine, a compound isolated from Veratrum californicum, is an antagonist of the sonic Hedgehog signaling pathway with anti-cancer effect in many types of cancer, including brain tumors. For these reasons, herbal medicines are considered to be very effective and, nowadays, more than 60% of cancer patients use alternative and complementary therapies. In a recent study published in 2012 by Ćurčić and collaborators, showed that methanol extract of Ligustrum vulgare, a specie native to Europe, North Africa and Western Asia, effectively destroye human colon cancer cells.

In vivo or in vitro anti-inflammatory, anti-mutagenic and anti-oxidant extract of L. vulgare have also been reported in the literature. The purpose of this study was to determine the volatile compounds L. vulgare hydroalcoholic extract (LHAE) and to analyze the effect of the extract on brain tumor cells. Using a panel of brain tumor cell lines derived from brain tumors, we analyzed the effect of LHAE as monotherapy or in combination with two of the most common chemotherapy used to treat brain cancer, TMZ and DOXO. In about 90% of glioblastomas cases arising de novo we detect the amplification of the epidermal growth factor receptor (EGFR). This overexpression of EGFR is often accompanied by loss of PTEN locus on chromosome 10q23. Using a glioblastoma cell line, in this study, EGFR was inhibited by AG556, in order to investigate the antitumor effect of receptor inactivation. Treatment with AG556 in concentrations of 1, 5 and 10 μM produced cytotoxicity in glioblastoma cells in a time dependent manner and concentration.

The study also determined the cytotoxic effect of combined treament (AG556 TMZ) on glioblastoma cells. AG556 and TMZ treatment produced a significant inhibition of the growth of glioblastoma cells GB1B in a dose-time dependent manner. Our results showed that cell line GB1B interaction between AG556 and TMZ therapy was synergistic at most of the combinations studied. In the study were also efctuat in vitro and in vivo experiments to evaluate the action of functionalized magnetic nanoparticles of $Fe_3O_4$/salicylic acid ($MNP_S$) on human glioblastoma. Following the studies, we observed that $Fe_3O_4$ nanoparticles had no significant effect on citotoxic human glioblastoma cells in vitro. For the in vivo study, human glioblastoma cells were

transplanted onto the chorioallantoic membrane (CAM) of the chicken embryo. $MNP_S$ were injected into the blood vessels of the CAM and guided to the tumor area with a strong static magnet. Thus there was a decrease in tumor xenograft growth due to necrotic lesions blocking intratumoral and peritumoral blood vessels induced by the accumulation of MNPs under the action of the magnetic field. This behavior suggests the potential of $Fe_3O_4$ magnetic nanoparticles to treat cancer and to encourage the use of these nanoparticles as drug carriers in the treatment of brain cancer. Currently, a study of ferrite nanoparticles loaded with Heliantin was conducted in our lab in order to assess their potential antineoplastic effect on brain tumor cells. Biological material such as tissue, cells, blood, serum, plays an important role in academic research. An important part of this project is to develop a brain tumor bank, providing a large number of archived biospecimen with related clinical and molecular data which supports fundamental clinical research. At this stage, tumor samples were collected from 60 patients with brain tumors by surgery at Department of Neurosurgery of Emergency Hospital "Bagdasar-Arseni", in order to expand the bank of brain tumors.

Statistical data processing: Statistical comparison of mean values was performed using the ANOVA t test. Results with p values $<0.05$ were considered statistically significant. All results are the mean ± standard deviation. The interaction between treatment with AG556 and TMZ was analyzed by multiplicative method (Chou TC et al. 1984) as follows:

- additive interaction occurs when $I1 + I2 = I1,2$;
- synergism occurs when $I > I1 + I2$; and
- antagonism occurs when $I1,2 < I1 + I2$.

**Articles:**

**BDI**

**1.** Plasma levels of Glucose and Insulin in patients with brain tumors. Oana Alexandru, Laurentiu Ene, Oana Stefana Purcaru, Daniela Elise Tache, Alisa Popescu, Oana Maria Neamtu, Ligia Gabriela Tataranu, Ada Maria Georgescu, Valerica Tudorica, Cornelia Zaharia and Anica Dricu· Current Health Sciences Journal, Vol. 40, No. 4, 2014

**ISI**

1. *In vitro* and *in vivo* effects of $Fe_3O_4$/salicylic acid magnetic nanoparticles on the human glioblastoma cells. S. A. Buteică, I. Mîndrilă, D.E. Mihaiescu, S.O. Purcaru, A. Dricu, C. Nicolicescu, J. Neamțu. Digest Journal of Nanomaterials and Biostructures. Vol. 9, No. 3. July – September 2014, p. 959-965

2. Cancer stem cells: biological functions and therapeutically targeting. Marius Eugen Ciurea, Ada Maria Georgescu, Stefana Oana Purcaru, Stefan-Alexandru Artene, Ghazaleh Emami, Mihai Virgil Boldeanu, Daniela Elise Tache, Anica Dricu· Int. J. Mol. Sci. *2014*, 15,

3. Rationale and *In Vitro* Efficacy of *Ligustrum Vulgare* Hydroalcoholic Extract for the Treatment of Brain Tumors George Dan Mogoşanu, Anica Dricu et al. PLOS ONE. Acceptet with major modifications, 2014

**International Conference presentations**

1. Stefana Oana Purcaru, Ada Maria Georgescu, Oana Neamtu, Daniela Elise Tache, Ligia Gabriela Tataranu, Vasile Ciubotaru and Anica Dricu. The cytotoxic effect of an EGFR tyrosine kinase Inhibitor on low-passage human brain tumour cultures in vivo, *ROM. J. BIOCHEM.*, 51, *Suppl.*, 1–112 (2014) The annual international conference of the SRBMB & Workshop ”Viral hepatitis from cell culture to clinic” June 5-6, 2014, Băile Felix, Oradea, Romania, pg 107

2. Ligia Tătăranu, Ştefana Oana Purcaru, G. D. Mogoşanu, Sandra Alice Buteică, Ada Maria Georgescu, Anica Dricu, The Emerging Role Of Alternative Therapy In Targeting Brain Tumours, The 40th Congress of the Romanian Society of Neurosurgery with International Participation, Bucharest, September 25th - 27th., 2014, pag 130

3. Ada Maria Georgescu, Ligia Gabriela Tataranu, Florentina Serban, Oana Alexandru, Vrajitoru Alisa, Roxana Folcuti, V. Ciubotaru and Anica Dricu, Egfr inactivation in combination with temozolamide induced synergistic cytotoxicity in low passage glioblastoma cell line: in vitro study , The 40th Congress of the Romanian Society of Neurosurgery with International Participation, Bucharest, September 25th - 27th., 2014, pag 129

**National Conference presentations**

1. Purcaru Ştefana Oana, Buteică Sandra Alice, Mogoşanu G. D., Tache Daniela Elise, Croitoru O., Dricu Anica, Efectul citotoxic al extractului hidroalcoolic din flori de ligustrum vulgare asupra

tumorilor cerebrale de grad înalt *in vitro*, Zilele UMF din Craiova, A XLIII-a ediţie 2014, 6-7 iunie 2014, ISSN 1843-2441, pag 23

2. Buteica Sandra Alice, Purcaru Ştefana Oana, Mogoşanu G. D., Tache Daniela Elise, Croitoru O., Dricu Anica, Extractul hidroalcoolic din flori de ligustrum vulgare potenţial tratament în astrocitomul de grad II, Zilele UMF din Craiova, A XLIII-a ediţie 2014, 6-7 iunie 2014, ISSN 1843-2441, pag 24

3. Tache Daniela Elise, Buteica Sandra Alice, Purcaru Ştefana Oana, Mogoşanu G. D., Croitoru O., Dricu Anica, Caracterizare linii celulare primare, Zilele UMF din Craiova, A XLIII-a ediţie 2014, 6-7 iunie 2014, ISSN 1843-2441, pag 25

4. Popescu Alisa Mădălina, Purcaru Ştefana Oana, Tătăranu Ligia, Dricu Anica, Gliomul malign recurent: prezentare de caz, Zilele UMF din Craiova, A XLIII-a ediţie 2014, 6-7 iunie 2014, ISSN 1843-2441, pag 30

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