Final Report
William Donald Nash Brain Tumour Research Fellowship,
Brain Tumour Foundation of Canada

Project title: Combi-molecule, ZRBA1, as a novel molecular targeted therapy integrated with high dose rate ionizing radiation to treat malignant glioma tumors
Project timing: July 2013 – July 2015
Research Fellow: Dr. Slawomir Kumala, Translational Radiation Oncology Laboratory, Jewish General Hospital, Segal Cancer Centre, McGill University, Montreal, QC, Canada

Project summary:

Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor in adult patients. The current standard of care comprises of maximum safe resection surgery followed by chemoradiation with Temozolomide (TMZ). However, a large percentage of tumors are resistant to the cytotoxic effects of TMZ due to the elevated expression of the repair protein O^6-methylguanin-DNA-methyltransferase (MGMT) and/or a defect in the mismatch repair (MMR) pathway. Additionally, among key elements involved in driving of glioblastoma growth is the transmembrane epidermal growth factor receptor (EGFR) which is known to be over expressed in 40-70% of GBMs and has been associated with aggressive tumor progression, invasion, and reduced sensitivity to chemoradiation. Therefore the main objective of these studies, as a part of the William Donald Nash Fellowship, was to develop a novel, targeted and more effective GBM treatment. I have been testing the radiosensitizing abilities of a novel binary targeting molecule, ZRBA1, designed to inhibit EGFR activity while additionally inducing DNA lesions of the similar class to TMZ. Collected data show that combination of this novel multi-targeting agent with radiation represents an extremely promising treatment option for GBM patients which has demonstrated to be very effective in subtypes of patients who acquired resistance to current therapy.

Project findings:

Collected data indicate that exposure of different GBMs cells to ZRBA1 before irradiation resulted in an increase in radiosensitivity of these cells with dose enhancement factors at surviving fraction of 0.1 ranging from 1.3 to 1.7. Additionally, such combinational treatment caused strong and long lasting cell cycle arrest in the G_2/M phase, which was accompanied by increased formation of DNA double-strand breaks (DSBs) as measured by the level of phosphorylated H2AX histone (γH2AX). Additionally, gene array experiments revealed a unique genetic signature for the combined treated group and demonstrated global upregulation of multiple DNA damage signaling and repair genes. Combination of ZRBA1 and radiation (IR) lead to an elevated senescence of GBM cells and their death as measured by induction of autophagy and apoptosis. In addition, ZRBA1 not only inhibits EGFR activity and its downstream signaling cascade but when combined with IR alternates phosphorylation pattern of different cell stress activated pathways as well as chaperone proteins.

As generally accepted, certain subpopulations of GBM cells are particularly prone to drive tumour progression and development of resistance against chemo- and radiotherapy. Those cells named cancer stem cells are also considered to be responsible for the

recurrence of brain tumors after existing therapies. Moreover, it is assumed that spheroid cultures of both, cell lines and patients derived specimens, can better predict the in vivo response than monolayer cultures, since cell-cell contact, variation in cell cycle, altered metabolism, and diffusion of nutrients, oxygen or drugs may influence the outcome of the therapy. Therefore, in the second phase of the project I was able to demonstrate that ZRBA1 is not only effective in a case of the established monolayer GBM cell lines but also radiosensitizes and inhibits the invasiveness of both three dimensional GBM's spheroids as well as neuropsheres derived from patient's brain tumor stem cells.

Importantly, in all of the above-mentioned GBM-models the effect of ZRBA1 treatment was identical in MGMT positive and negative cells/spheres/neurospheres as well as independent of overexpression or mutation of EFGR. Thus, in contrast to Temozolomide radio-sensitizing proprieties of ZRBA1 do not depend on the factors that limit the use of current GBM targeted therapies.

Overall, our findings suggest that ZRBA1 binary targeting molecule which possesses the ability to enhance the levels of DNA damage, inhibits DNA repair and increases cell death could be developed as a novel and unique radiosensitizing agent to treat GBM.

All of these studies are part of a manuscript which is currently under preparation. In addition, in order to explore the efficacy of this promising treatment in vivo, an intracranial mouse model of GBM is currently being evaluated. Our initial data indicate that ZRBA1 is particularly suitable for the treatment of GBM because of its ability to cross the brain-blood barrier (BBB). Also metabolomics studies have been initiated in order to investigate the exact mechanism of action of this promising combined modality treatment.

Significance of the Fellowship:

William Donald Nash Brain Tumour Research Fellowship greatly helped me to advance my knowledge and gain a new experience in the area of brain tumors research. Moreover, it had given me a unique opportunity to stimulate development of my scientific career and refine my research, analytical and organizational skills. Leading this project provided me with the opportunity to collaborate with the McGill Drug Development Laboratory, Dr. A. Luchman from D. Weiss laboratory at the University of Calgary or Dr. J. Rak laboratory at the McGill University. This funding has also allowed me to participate in several other studies (please see the attached list of publications/communications) as well as to attend international and local scientific meetings and present my data in a front of high-level scientists and physicians dedicated to all aspects of GBM treatment. Furthermore, majority of the above mentioned data has become a "research base" for applying for external governmental research funding to continue this promising project.

I am very thankful and would like to express my deepest gratitude to William Donald Nash Family and Brain Tumour Foundation of Canada for the funding. Without their precious support it would not be possible to conduct this research project, which I believe will help us bring this promising therapy closer to the clinical application. Thank you!

Sincerely

Slawomir Kumala

Publications per reviewed and in preparation:

1. **Kumala S.**, Heravi M., Devic S. Sadr MS., Jean-Claude BJ., Del Maestro R., Muanza T., "Novel binary alkylator and EGFR inhibitor for chemo-radiation of malignant glioma" (in preparation).
2. Ghashghaei M., Niazi T., **Kumala S.**, Bekerat H., Paliouras M., Muanza T., "Interaction of Enzolutamide and ionizing radiation: radiosensitization of prostate cancer through androgen receptor inhibition" (in preparation)
3. Maria O., **Kumala S.**, Heravi M., Syme A., Eliopoulos N., Muanza T., "Irradiation of Adipose Mesenchymal Stem Cells induces a rapid onset and early resolution of DNA repair", Cytotherapy 2015 (accepted)
4. Heravi M*, **Kumala S***, Rachid Z., Jean-Claude BJ., Radzioch D., Muanza TM., "ZRBA1 a mixed EGFR/DNA targeting molecule potentiates radiation response through delayed DNA damage repair process in a Triple Negative Breast Cancer Model", Int J Radiat Oncol Biol Phys. 2015 Jun 1;92(2):399-406. *(*equal contribution)*

Communications:

1. Muanza T., **Kumala S.**, Heravi M, Bekerat H., Sadr MS., Del Maestro R., Jean-Claude B., Novel Binary Molecule, ZRBA1, as unique MGMT independent radiosensitizer to treat malignat gliomas, 29th CARO Annual Scientific Meeting September 9-12, 2015 Kelowna, BC
2. **Kumala S**, Heravi M, Bekerat H, Sadr MS, Jean-Claude BJ, Del Maestro R, Muanza T, Novel binary targeting molecule enhances radiation response in glioma model by induction of DNA damage and delay of DNA repair, McGill CIHR-Drug Development Training Program Workshop, 28th November 2015, Montreal, Canada
3. **Kumala S.**, Heravi M., Devic S., Sadr MS., Del Maestro R., Muanza T. Novel binary targeting molecule enhances radiation response in glioma model by induction of DNA damage and delay of DNA repair. 16th Biennial Canadian Neuro-Oncology Meeting, 12-14 June 2014, Halifax, Nova Scotia.
4. Heravi M., **Kumala S.**, Rachid Z., Jean-Claude BJ., Radzioch D., Muanza T. Potentiation of radiation response by a novel EGFR/DNA targeting molecule in a triple negative breast cancer model. International Conference on Translational Research in Radiation Oncology, 10-14 February 2014, Geneva, Switzerland.
5. **Kumala S.**, Heravi M., Devic S., Maria OM., Alshami J., Sadr MS., Del Maestro R., Muanza TM. Novel EGFR/DNA binary targeting molecule ZRBA1 potentiates radiation response in glioblastoma tumour model, l4th Quadrennial Meeting of the World Federation of Neuro-Oncology, 21-24 November 2013**,** San Francisco, USA
6. Maria O., **Kumala S.**, Heravi M., Eliopoulos N., Muanza T. Adipose-Derived Mesenchymal Stromal Stem Cells have superior Radiation-Induced DNA damage repair machinery. CCRA Annual Conference, 3-6 November 2013, Montreal, Canada
7. Heravi M., **Kumala S.**, Rachid Z., Jean-Claude BJ., Radzioch D., Muanza T. EGFR/DNA targeting molecule potentiates radiation response in murine breast cancer model. ASTRO 55th Annual Meeting 2013, 22-25 September 2013, Atlanta, USA