1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan, all in English)

Project title: Purinergic signaling in glioma – *in vitro* studies on pathological mechanisms and therapeutic potential

1.1. Project goals

The main aim – disclosing the potential of purinergic compounds in human glioma therapy, will be achieved by below listed particular goals:

- screening of different human glioma cell lines and cell cultures derived from patients in terms of purinergic signaling influence on their proliferation and differentiation
- controlled induction of glioma cells differentiation using purinergic compounds (nucleotides, nucleosides, receptors and enzymes)
- assessing the *in vitro* effects of tailored, combined chemotherapy based on the use of purines together with temozolomide and retinoic acid in glioma patients samples

1.2. Outline

Gliomas represent the most aggressive and difficult to treat brain tissue primary tumors that originate from glial cells [1]. Surgical interventions in brain are extremely risky, while the presence of the blood-brain barrier drastically limits the list of effective chemotherapeutics. Temozolomide is currently the most commonly used drug, but the long-term temozolomide monotherapy leads to selective pressure and survival of resistant cancer cells [1,2]. These aggressive and low differentiated tumor stem cells with increased proliferative potential are the main reason for the inefficiency of glioma treatment. **One of the currently evaluated strategies is the controlled differentiation of proliferating glioma cells**. The synchronization of cell cycles within the tumor and decrease in their proliferative potential can be promoted by the use of retinoic acid [3]. This drug used in combination with temozolomide was reported to give synergic therapeutic effect; unfortunately, not without severe side effects [4].

Purinergic signaling components (nucleotides, nucleosides, receptors, enzymes) contribute significantly to the modulation of physiological and pathological processes as cell proliferation, differentiation, inflammation, immune response, and apoptosis in all human cells and tissues [5,6]. Recently, they have emerged as important factors underlying cancer cells invasive potential. Thus, **purinergic pathways controlling cell proliferation, survival and death can be effectively harnessed as targets for purine-based anti-cancer therapy**. Several studies showed the inhibitory

effect of ATP on the proliferation of cancer cells of the intestine, esophagus, skin, lungs, cervix, prostate, bladder, glia or melanocytes [7]. On the other hand, the excessive amount of ATP released from damaged cells during radio- or chemotherapy can increase the rate of tumor cell proliferation, and promote the formation of a metastatic microenvironment [7,8]. Antagonists of purinergic receptors as well as some ecto-enzymes (nucleotidases) can inhibit ATP-dependent processes [9]. However, reducing the concentration of ATP in the tumor environment leads to adenosine production, that triggers permanent immunosuppression and promotes metastasis. The lack of unambiguous results suggest that **the effect of purines on cancer cells may be associated with cancer type or even depend on individual tumor features**, and thorough studies are still required in this field.

We assume that purinergic compounds could also be exploited to overcome glioma cells resistance to chemotherapy and to increase the efficiency of pharmacological approach. Preliminary results of our research group indicate that nucleotides (ATP) and P2X7 receptor agonists (e.g. bzATP) **increase the efficiency of neurogenic differentiation and are potent modulators of cell proliferation** [10,11]. The lowered expression of P2X7 receptor in some gliomas is responsible for their resistance to ATP-induced cell death, and hypoxic conditions in tumor niche upregulate adenosine P1 receptors, promoting proliferation and migration of glioma cells [12,13]. As the pleotropic effects of purines seem to be dependent on cellular context and microenvironment, **the promising purine-based therapeutic approach must be thoroughly studied *in vitro***. The meticulous screening of different human glioma cell lines and cell cultures derived from patients is required to disclose specific pathological mechanisms and therapeutic potential of purinergic signaling in glioma.

1.3. Work plan

- *In vitro* culture of glioma cell lines and samples derived from patients
- Molecular and biochemical characterization of purinergic compounds in glioma cells
- Assessment of purinergic signaling influence on cell proliferation and differentiation
- Evaluation of differentiation efficacy in glioma cultures through molecular markers expression analyses
- Functional tests (viability, apoptosis, invasive properties) confirming the influence of purinergic compounds combined with chemotherapy on glioma

1.4. Literature

1. Taal W, Bromberg JEC, van den Bent MJ. Chemotherapy in glioma. CNS Oncol 2015, 4: 179-192.
2. Lee SY. Temozolomide resistance in glioblastoma multiforme. Genes Dis 2016, 3: 198-210.

3. Rhinn M, Dollé P. Retinoic acid signaling during development. Development 2012, 139: 843-858.
4. Shi L, Li H, Zhan Y. All-trans retinoic acid enhances temozolomide-induced autophagy in human glioma cells U251 via targeting Keap1/Nrf2/ARE signaling pathway. Oncol Lett 2017, 14: 2709-2714.
5. Burnstock G. Purinergic signalling and disorders of the central nervous system. Nat Rev Drug Discov 2008, 7: 575-590.
6. Burnstock G. Purine and pyrimidine receptors. Cell Mol Life Sci 2007, 64: 1471-1483.
7. Rapaport E. Treatment of human tumor cells with ADP or ATP yields arrest of growth in the S phase of the cell cycle. J Cell Physiol 1983, 114: 279-283.
8. Schneider G, Glaser T, Lameu C, Abdelbaset-Ismail A, Sellers ZP, Moniuszko M, Ulrich H, Ratajczak MZ. Extracellular nucleotides as novel, underappreciated pro-metastatic factors that stimulate purinergic signaling in human lung cancer cells. Mol Cancer 2015, 24: 201.
9. Burnstock G, Di Virgilio F. Purinergic signalling and cancer. Purinergic Signal 2013, 9: 491-540.
10. Czarnecka J, Porowińska D, Bajek A, Hołysz M, Roszek K. Neurogenic differentiation of mesenchymal stem cells induces alterations in extracellular nucleotides metabolism. J Cell Biochem 2017, 118: 478-486.
11. Roszek K, Makowska N, Czarnecka J, Porowińska D, Dąbrowski M, Danielewska J, Nowak W. Canine adipose-derived stem cells: Purinergic characterization and neurogenic potential for therapeutic applications. J Cell Biochem 2017, 118: 58-65.
12. Liu J, Li N, Sheng R, Wang R, Xu Z, Mao Y, Wang Y, Liu Y. Hypermethylation downregulates P2X7 receptor expression in astrocytoma. Oncol Lett 2017, 14: 7699-7704.
13. Braganhol E, Wink MR, Lenz G, Battastini AMO. Purinergic Signaling in Glioma Progression. Adv Exp Med Biol 2020, 1202: 87-108.

1.5. Required initial knowledge and skills of the PhD candidate

- knowledge on biochemistry, cytophysiology and cell culture
- basic understanding of chemistry
- understanding of biochemical and molecular biology techniques
- analytical thinking and skills
- open to challenging tasks and creative
- hard-working person, eager to learn

1.6. Expected development of the PhD candidate's knowledge and skills

- innovative thinking
- ability to plan and organize laboratory work
- skilled in novel scientific techniques
- ability to solve research problems
- ready to work in international research group