Glioblastoma multiforme (GBM) is the most common malignant brain cancer with a very poor prognosis and a median survival of approximately 10 months. Existing therapy, unfortunately, has very limited efficacy as cancer cells recur in 90% of cases. New therapeutic approaches of GBM treatment are desperately needed. One of the promising novel regimens may be immunotherapy – a type of cancer treatment that exploits the potential of immune system cells, in particular, cytotoxic T lymphocytes (CTLs), to find and destroy tumor cells. Among the immune cell subsets that demonstrate antitumor activity, innate-like T cells that carry T cell receptors (TCR) composed of γ and δ chains (γδ T cells) are of particular interest. In humans, there are two major populations of γδ T cells which were shown experimentally to exert anticancer functions in various settings: $V\delta2^+$ cells – dominant in the blood and $V\delta1^+$ cells – mostly located in peripheral tissues. It has been shown that $V\delta2^+$ cells recognize phosphoantigens derived from the mevalonate pathway of mammalian cells, which is highly active upon cell infection or oncogenic transformation, while $V\delta1^+$ cells respond to the major histocompatibility complex class I-related molecules A and B and UL16-binding proteins expressed on many stressed and tumor cells. The mechanisms of ligand binding by γδ TCR and cell activation are still not fully understood, hence, it is not clear why in certain cancers γδ T cells switch from cytotoxic to regulatory and tumor-promoting activity. For instance, it has been demonstrated in patients with glioma that $V\delta1^+$ cells develop an immunosuppressive phenotype. However, with a proper in vitro expansion protocol, $V\delta1^+$ cells can be turned into very efficient killers fit for immunotherapeutic applications in humans. So far, in preclinical studies in mice, only the in vitro expanded $V\delta2^+$ cells have been tested for GBM therapy with some success, while $V\delta1^+$ cells were analyzed in a chronic lymphocytic leukemia model showing the capability for tumor growth inhibition. The available data indicate that intracranial administration of in vitro expanded $V\delta2^+$ cells into immunodeficient mice with brain GBM tumors leads to the killing of cancer cells and tumor shrinkage, thereby proving the potential of γδ T cells for the GBM immunotherapy. In those experiments, however, the tumor microenvironment (TME) lacked other human leukocytes which otherwise could affect the therapeutic outcomes. For instance, regulatory T cells usually infiltrate tumors and suppress immune responses against cancer. Also, effector functions of CTLs diminish in time due to exhaustion mediated by T-cell-expressed inhibitory receptors upon binding cognate ligands present on surrounding cells in the TME. Thus, it is not known whether the cytotoxicity of γδ T cells toward GBM cells, as shown by others in the immunodeficient mouse model, is effective in an immunocompetent GBM microenvironment. Addressing this knowledge gap is critical for recognizing the true translational potential of γδ T cells in GBM. Glucocorticoids are another important factor in the context of GMB immunotherapy. They are used in the palliative treatment of brain as relief from increased intracranial pressure and brain edema. As a side effect they lower the number of immune cells (including γδ T cells) in the blood of GBM patients and may also affect TCR signaling, thus, the efficacy of γδ T cell immunotherapy in such a scenario can be strongly affected.

With all this in mind, we propose to compare effector functions of in vitro expanded $V\delta1^+$ and $V\delta2^+$ cells toward GBM cells to determine which subset suits best for immunotherapeutic applications as it has not been investigated yet. Thanks to a collaboration with neurosurgeons, we will have freshly resected brain tumors for the isolation of GBM cells. We will also characterize the in vivo cytotoxic activity of γδ T cells modulated by immunosuppressive human GBM microenvironment. To this end, we will perform co-transplantation of patient's immune system cells and tumor cells into immunodeficient mice. The use of such "humanized" mice will enable in vivo studies of γδ T cell immunotherapy within more human-like GBM TME than in less advanced systems. Thus, the modulation of γδ T cell activity by the humanized TME will be scrutinized for the first time. Notably, we will not only look at tumor growth inhibition but also employ intravital imaging to study the γδ T cell dynamics within the TME. We will perform high-resolution microscopic visualization of γδ T cells in real-time showing their migration pattern and cellular interactions in the TME. By means of live imaging, we will determine whether direct contacts with GBM or other cells surrounding the tumor are necessary to observe cancer killing. This type of analysis is unprecedented up to now. Finally, we will examine how glucocorticosteroids, being a standard treatment during the post-operative GBM therapy, affect the functions of normal versus glucocorticosteroid receptor-deficient γδ T cells within the TME, which topic has not been studied before. Ultimately, the knowledge acquired in the course of this project will have a significant positive impact on the development of new strategies for the treatment of GBM such as immunotherapy with γδ T cells.