# Development and Characterization of Two Novel Nanomedicine-based Approaches to Restore the Anti-tumor Activity of the Immune System in Glioblastoma Patients

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The tumor microenvironment (TME) plays a pivotal role in cancer progression and treatment response. Glioblastoma multiforme (GBM), is characterized by a highly immunosuppressive TME, that contains a variety of non-neoplastic cells, including a considerable proportion of infiltrating leukocytes, most of which are macrophages representing around 30% of the total tumor mass. Targeting specific cell subsets within the TME in glioblastoma multiforme (GBM), can indeed be beneficial, but it poses several challenges due to the unique characteristics of the brain and the complexity of the TME. Our recent studies highlight that bone marrow-derived macrophages (BMDMs), distinct from resident microglia, accumulate towards the core of GBM lesions and exhibit potent immune suppressive activity [1].
Given the peculiarity of GBM microenvironment, advances in nanotechnology, immunotherapy, and targeted drug delivery systems may offer promising avenues for developing more effective and precise treatments for GBM. Indeed, in our project we combined both the fields of nanomedicine and tumor immunology and developed two novel approaches to modulate the immune response in GBM patients by exploiting innovative controlled drug delivery nanosystems. One is an Oil-in-Water (O/W) nanoemulsion (NEs), prepared using the microfluidic technique. This NE is loaded with Zinc protoporphyrin IX (ZnPPIX), a potent inhibitor of heme oxygenase-1 (HO-1), an enzyme involved in the iron metabolism and immunosuppressive activity of BMDMs [2].
The second nanosystem is a polymeric nanoparticle, prepared using the ionic gelation technique. It is loaded with a derivative of the chemotherapeutic drug oxaliplatin called diaminocyclohexane-platinum II (DACHPt) [3], capable of inducing immunogenic cell death (ICD), a phenomenon that can be detected through the measurement of specific danger-associated-molecular-patterns (DAMPs) like the extracellular ATP and HMGB1 release [4]. Both nanosystems were characterized for their physicochemical properties.
Our results obtained through *in vitro* models and primary cells present in the TME of GBMs, suggest that the ZnPPIX-loaded NEs could be used to target BMDMs and induce their re-programming towards a more pro-inflammatory and anti-tumoral phenotype. Moreover, data regarding the polymeric nanosystem showed that it could be exploited to target both tumor cells and myeloid immunosuppressive cells while inducing ICD.

**References**

[1] L. Pinton *et al.*, (2019) "The immune suppressive microenvironment of human gliomas depends on the accumulation of bone marrow-derived macrophages in the center of the lesion," *J. Immunother. Cancer*, vol. 7, no. 1, p. 58
[2] S. Magri *et al.*, (2022) "The immunosuppression pathway of tumor-associated macrophages is controlled by heme oxygenase-1 in glioblastoma patients," *Int. J. Cancer*, vol. 151, no. 12, pp. 2265–2277
[3] K. Matha *et al.*, "Bioinspired hyaluronic acid and polyarginine nanoparticles for DACHPt delivery," *Eur. J. Pharm. Biopharm.*, vol. 150, pp. 1–13
[4] J. Fucikova *et al.*, (2019) "Detection of immunogenic cell death and its relevance for cancer therapy," *Cell Death Dis.*, vol. 11, no. 11, p. 1013