Novels drug delivery nanosystems to restore the anti-tumor activity of the immune system in cancer patients

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Background

Glioblastoma multiforme (GBM) is the most prevalent and challenging primary brain tumor to treat, known for its highly immunosuppressive tumor microenvironment (TME). This unique TME can be harnessed to develop innovative approaches for targeting specific cancer cell populations, particularly focusing on tumor-associated macrophages (TAMs), which play a central role in suppressing the anti-tumor immune response in GBM and contributing to a poor prognosis [1]. These TAMs consist of both resident microglia (MG) and macrophages originating from the bloodstream (bone marrow-derived macrophages, BMDMs). In 2019, our group has demonstrated that BMDMs are especially abundant in the core of GBM tumor masses, where they exhibit strong immunosuppressive activities [2].

Given these peculiar features of the TME, in this study we combined both the fields of nanomedicine and tumor immunology and proposed a novel approach to modulate the immune response in GBM patients by exploiting two controlled drug delivery nanosystems.

One is an Oil in Water (O/W) nanoemulsion that contains a potent inhibitor of heme oxygenase-1 (HO-1), Zinc protoporphyrin IX (ZnPPIX). We selected this inhibitor based on our recent research findings, which showed that the immunosuppressive activity of TAMs is primarily linked to their iron metabolism [3].

The other nanosystem is a polymeric nanoparticle loaded with an oxaliplatin derivate, diaminocyclohexane-platinum II (DACHPt) [4]. The use of oxaliplatin derivates trigger immunogenic cell death (ICD) within the TME of glioblastoma patients.

Methodolody

ZnPPIX loaded nanoemulsions (NEs) were produced via microfluidic technique, while DACHPt-loaded hyaluronic acid-polyarginine nanoparticles were prepared using the ionic gelation technique at LAGEPP laboratory, in Lyon [3,4].

Immunosuppressive activity of *in vitro*-derived macrophages was assessed by evaluating the proliferation of activated lymphocytes that were stained with CellTrace™ Violet Cell Proliferation Kit, activated with anti-CD3 and anti-CD28 and co-cultured in 96 plate with macrophages. After 4 days, proliferation of T cells was evaluated through the signal of CellTrace on $CD3^+$ cells, by flow cytometry. Cell viability assay were carried on GBM cell line (U87MG) to determine the targeting efficacy and safety of polymeric nanoparticles (NPs).

Results

We developed NEs loaded with ZnPPIX and invastigated their physico-chemical characteristics. Our results demostrated that NE-ZnPPIX presented a size of around 100 nm, a slightly negative zeta potential of -10 mV and an encapsulation efficiency (EE) of the drug of around 69% (figure 2). Immunesuppressive assay have demostrated that treatement with either ZnPPIX free drug or NE-ZnPPIX relief the immunesuppression activity of macrophages and promote the proliferation of the co-coltured lymphocytes (Figure 3, left panel).

In parallel we also developed DACHPt-loaded nanoparticles (NPs) that show a size of around 200 nm with a negative Zeta Ponteial (-20 mV). Also in this case the EE was high (around 70%).

Studies on GBM cell line showed a targeting specificity of DACHPt-loaded NPs as doses increased and blank NP did not interfere with cell viability at all the concentrations tested, confirming their biocompatibility and safety usage for a local and systemic treatment (figure 3,right panel).

Conclusions

Our results obtained through the *in vitro* models and our findings regarding the uptake of these nanosystems by cells present in the TME of glioblastoma (data not shown), suggest that the ZnPPIX loaded NEs could be used to target TAMs and induce their re-programming towards a more pro-inflammatory and anti-tumoral phenotype. In addition, our results indicate that the polymeric nanosystem could be used to target both tumor cells and myeloid immunosuppressive cells while inducing ICD (figure 4).

References

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Figures

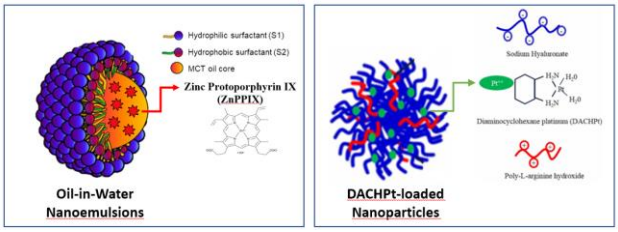

Figure 1. Schematic representation of the two nanosystem used for this study (*Adapted from [3,4]).*

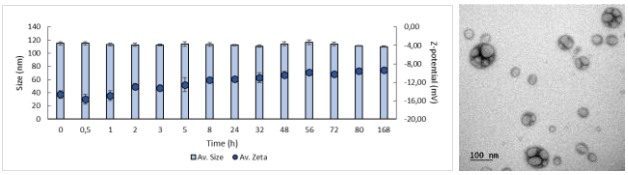

Figure 2. Characterization of NEs: Dynamic-Light-Scattering and Zeta-potential measurements indicate NEs average size around 110 µm, PDI < 0.2, zeta-potential values around -10 mV; TEM image showed a well-defined and homogeneous structures with spherical shape of NEs.

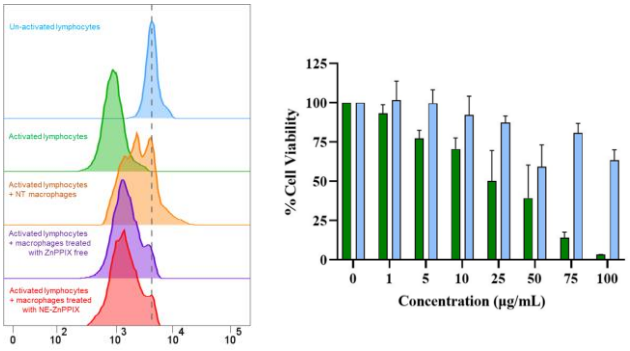

Figure 3. *Left panel:* Proliferation of activated T cells in the presence of untreat or drug-loaded NEs, a representative flow cytometry analysis of the immunosuppressive assay showing the efficacy of NE-ZnPPIX and that of the ZnPPIX free drug in restoring the proliferation of activated lymphocytes. *Right panel:* cell viability assay on U87MG cells in the presence of the polymeric nanosystem (empty NPs in light blue, DACHPt-loaded NPs in green).

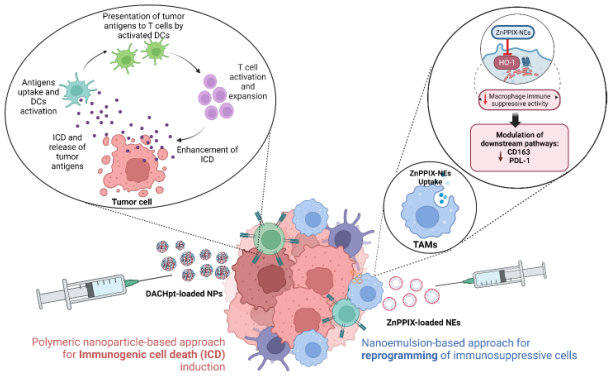

Figure 4. Proposed mechanisms of action of the two encapsulated nanosystems to restore the anti-tumour activity of the immune system in cancer.

Acknowlegments

This research was supported by *Arqus European University Alliance*.