Dual Strategy for Glioblastoma Treatment: Targeted Photothermal Ablation and Macrophage-Mediated Nanoparticle Distribution

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Introduction

Glioblastoma (GBM) remains the most aggressive and lethal brain tumor, characterized by limited treatment efficacy and recurrence driven by CD133+ Cancer Stem Cells (CSCs). Successful clinical translation of gold nanorod (GNR)-mediated Photothermal Therapy (PTT) requires substantial improvements in targeting specificity and uniform intratumoral nanoparticle (NP) distribution. This study evaluates a dual strategy to address these critical barriers. First, we demonstrated enhanced targeted photothermal ablation using GNRs biofunctionalized with anti-CD133 antibodies. This active targeting allowed for parameter optimization in CT2A cells, achieving sustained high therapeutic efficacy (~85% cell death) despite a reduction in laser power (to 3 W) and GNR dose (to 2 µg/mL). Second, we leveraged macrophages as cellular vectors due to their innate tumor-tropic behavior, ensuring improved dispersion and penetration into the tumor microenvironment. This combined approach not only enhances local ablation but also promotes Immunogenic Cell Death (ICD), transforming local therapy into a potentially systemic anti-tumor immune response. These results support the development of a safer and more effective PTT modality for GBM.

Material, methods and Statistics

This dual strategy employed Gold Nanorods (GNRs) targeted via a novel construct combining Protein G-anti-CD133 antibodies to eliminate glioblastoma stem-like cells (CT2A). Successful biofunctionalization was verified by Dynamic Light Scattering (DLS) and Zeta Potential analysis, which confirmed the expected increase in hydrodynamic diameter (from 102 nm to 124 nm) and the shift in surface charge. Photothermal therapy (PTT) efficacy was maximized, allowing a reduction of the standard parameters (4.5 W, 3 µg/mL) to 3 W laser power and 2 µg/mL GNR concentration, maintaining therapeutic outcomes. To enhance spatial distribution, murine macrophages (RAW 264.7) were used as cellular vectors, exploiting their innate tumor-tropic behavior. Optimization studies established the most efficient loading protocol was direct incubation, and a 4:1 macrophage-to-tumor cell ratio (B16-F10 co-culture) was selected. PTT was conducted using an 808 nm laser at 3 W for 10 minutes. Cell viability was quantified using XTT assays and visually confirmed with Calcein/PI dual staining. Finally, immunofluorescence protocols were standardized to assess Immunogenic Cell Death (ICD) potential by detecting Calreticulin (CRT) expression.

Targeted Photothermal Ablation and Parameter Optimisation (GBM Model)

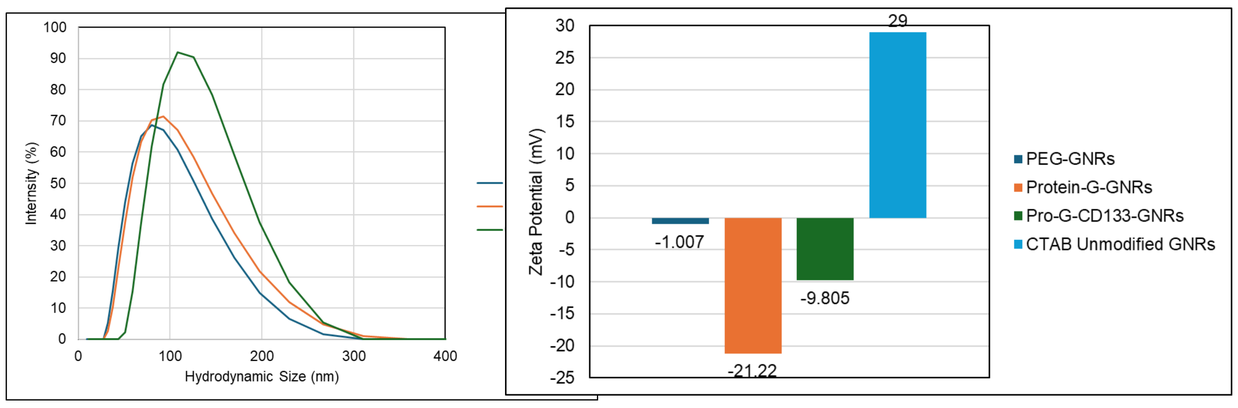

Figure 1: DLS and Zeta Potential: Dynamic Light Scattering (DLS) verified the sequential increase in hydrodynamic diameter from Protein G-GNRs (102 nm) to CD133-GNRs (124.1 nm). This was supported by a shift in the zeta potential from –21.2 mV (Protein G-GNRs) to –9.8 mV (Pro-G-CD133-GNRs), confirming antibody attachment.

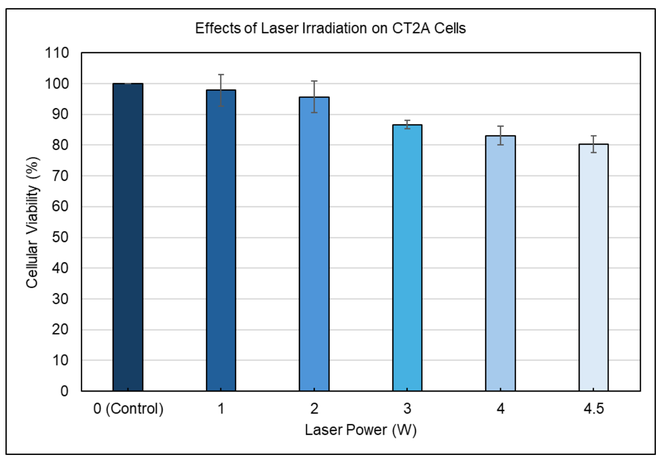

Figure 2: Ablation Efficiency: Under standard conditions (4.5 W laser, 3 µg/mL GNRs), the CD133-GNRs exhibited the highest cytotoxicity (86% cell death) in CT2A glioblastoma cells, establishing a strong therapeutic benchmark (viability of 14.2%)

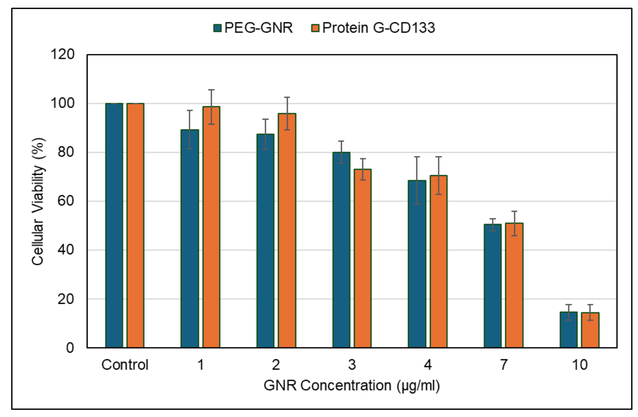

Figure 3: PTT, 4.5W for 10 minutes mediated with the different GNRs all at concentrations of 3 µg/mL. (standard PTT conditions used).

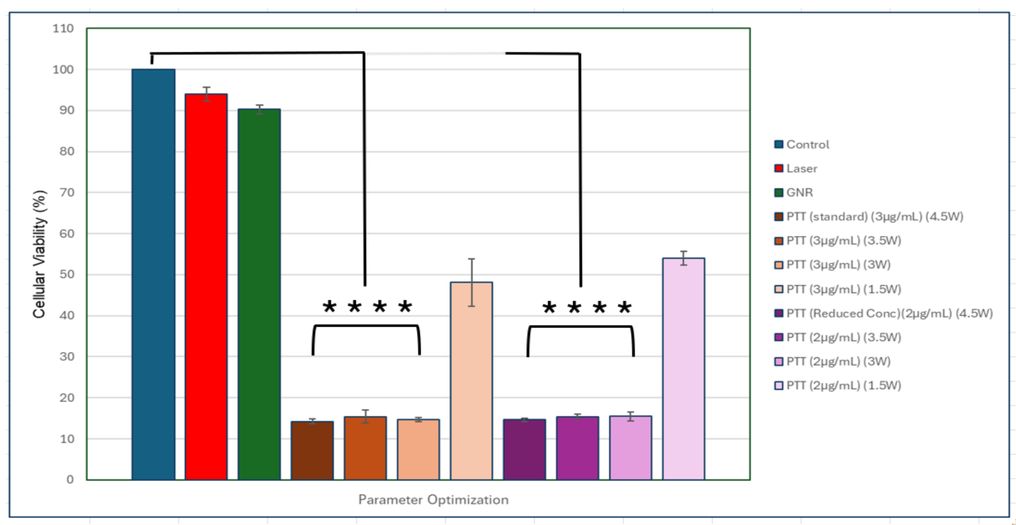

Figure 4: Optimization of CD133-Targeted PTT: XTT Results.

Macrophage-Mediated Nanoparticle Distribution (Melanoma Co-culture)

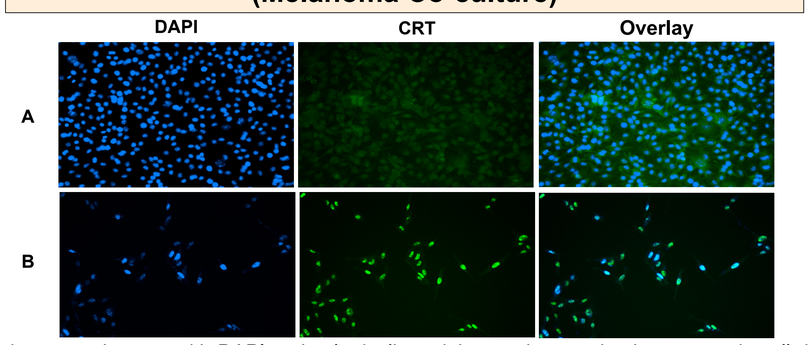

Figure 5: Fluorescence microscopy images with DAPI and calreticulin staining to characterize immunogenic cell death caused by the optical hyperthermia experiment with 3 W laser power and [2µg/mL] AuNPs in melanoma cells. **A.** Control cells, CRT is not overexpresed. **B.** optical hyperthermia experiment, images taken at 20x magnification and CRT antibody was left for 1 hour. Staining with CRT shows an increase in intensity, which means its presence on the cell surface.

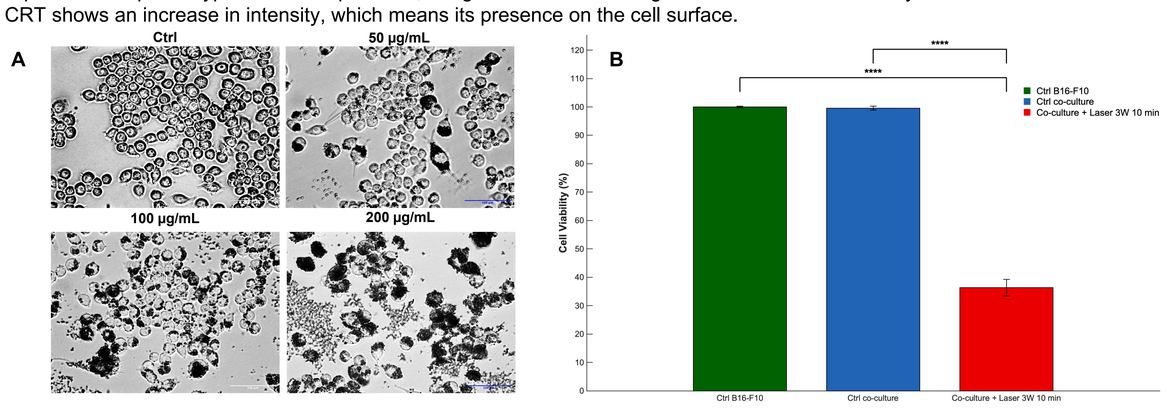

Figure 6: A. Transmision light images showing the uptake of AuNRs and FeMPs at different concentrations by RAW 264.7 cells with the ultimate goal of focusing macrophages to the space of interest. **B.** Cell viability in co-culture of AuNP-loaded macrophages and melanoma cells after 3W laser for 10 min

Conclusions

1. CD133-targeted GNRs demonstrated superior photothermal efficiency, achieving the highest cytotoxicity (86%) in CT2A glioblastoma cells under baseline conditions.
2. Active targeting enabled significant PTT parameter optimisation, sustaining high efficacy (85% cell death) at reduced laser power (3 W) and lower GNR concentration (2 µg/mL).
3. RAW 264.7 macrophages have been shown to efficiently internalise iron and gold nanoparticles, thus confirming their viability as cellular vectors to enhance distribution within tumour tissue and facilitate their spatial distribution to the tumour area.
4. Macrophage-mediated optical hyperthermia in the optimized 4:1 co-culture system significantly reduced B16-F10 melanoma cell viability by 65% (remaining viability of 36.3%).
5. Nanoparticle-induced OH successfully activated Immunogenic Cell Death (ICD), confirmed by the overexpression and membrane exposure of the biomarker Calreticulin (CRT).
6. This dual strategy establishes a safer and more effective PTT protocol for GBM, integrating molecular precision with enhanced nanoparticle delivery and immune stimulation.

Bibliography

1. Vines, J.B.; Yoon, J.-H.; Ryu, N.-E.; Lim, D.-J.; Park, H. Gold Nanoparticles for Photothermal Cancer Therapy. Front. Chem. **2019**,7, 167.
2. Casanova-Carvajal, O.; Urbano-Bojorge, A. L.; Ramos, M.; Serrano-Olmedo, J. J.; Martínez-Murillo, R. Slowdown intracranial glioma progression by optical hyperthermia therapy: Study on a CT-2A mouse astrocytoma model. Nanotechnology 2019,30, 355101.
3. Poonaki, E.; et al. CD133-Functionalized Gold Nanoparticles as a Carrier Platform for Telaglenastat (CB-839) against Tumor Stem Cells. Int J Mol Sci 2022,23, 5479.
4. Im, N. R.; et al. Application of M1 macrophage as a live vector in delivering nanoparticles for in vivo photothermal treatment. J Adv Res 2021,31, 155.
5. Garg, A. D.; et al. Trial watch: Immunogenic cell death induction by anticancer chemotherapeutics. Oncoimmunology 2017,6, 1386829.

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