Enhanced In Vivo Efficacy and Safety of Combination Temozolomide and Bromodomain Inhibitor Therapy for Gliomas Using a Targeted Dual Drug-loading Stealth Liposomal Carrier

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Introduction

Development of rapid treatment resistance to single agent therapies and the presence of the blood-brain barrier (BBB) have limited significant improvements in outcomes for patients with gliomablastomas (GBM). To address these issues, we developed dual-drug loaded transferrin-functionalized nanoparticles containing the novel combination of temozolomide and the bromodomain inhibitor JQ1 and tested their safety and efficacy in U87MG and GL261 intracranial orthotopic GBM mouse models.

Methods

In vivo multiphoton imaging through a cranial window was performed to assess tumor uptake of nanoparticles. Mice containing U87MG or GL261 intracranial orthotopic tumors stable expressing GFP and luciferase were treated for 5 consecutive days with IV injections of either free drug or drug-loaded Tf-NPs. Tumor response to treatment was tracked every 3 to 5 days using bioluminescence imaging. Serial daily blood profiling was performed to assess systemic drug toxicity. Brains were harvested, fixed, and stained to detect markers of DNA damage (gamma-H2AX), apoptosis (cleaved caspase 3), and proliferation (Ki67) following treatment.

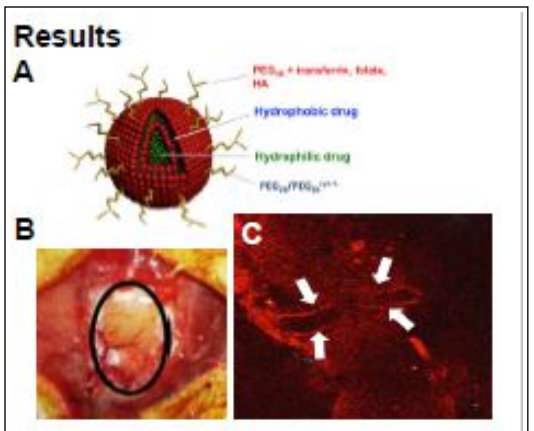

Figure 1. Dual drug-loading transferrin-functionalized liposomes (Tf-NP) cross the intact blood-brain barrier in mice. A) Schematic of PEGylated liposome. B) Cranial window (black oval delineating craniotomy). C) Composite multiphoton image showing accumulation of Tf-PEG2K-Cy5.5 NPs in the endothelial wall of a brian microvessel (white arrows) with diffusion across the BBB and aggregation of NPs in the surrounds brain milieu.

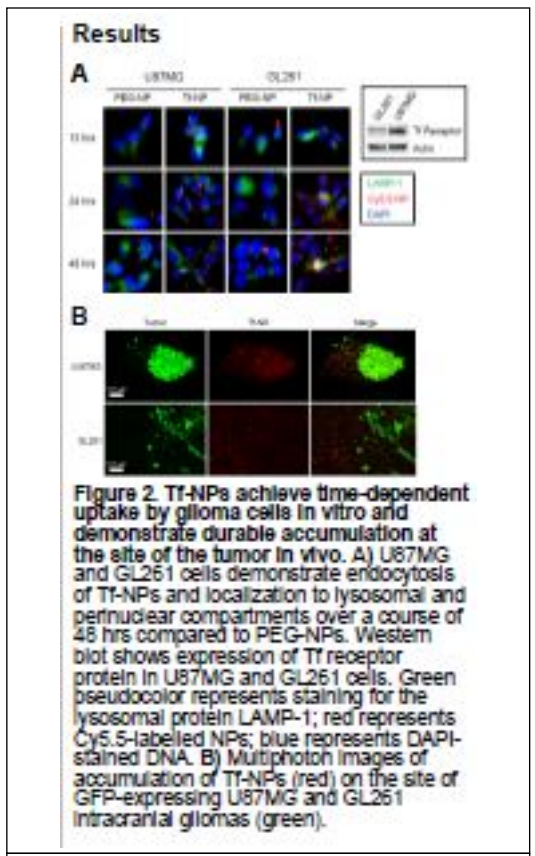

Figure 2. Tf-NPs achieve time-dependent uptake by glioma cells in vitro and demonstrate durable accumulation at the site of the tumor in vivo. A) U87MG and GL261 cells demonstrate endocytosis of Tf-NPs and localization to lysosomal and perinuclear compartments over a course of 48 hrs compared to PEG-NPs. Western blot shows expression of Tf receptor protein in U87MG and GL261 cells. Green pseudocolor represents staining for the lysosomal protein LAMP-1; red represents Cy5.5-labelled NPs; blue represents DAPI-stained DNA. B) Multiphoton images of accumulation of Tf-NPs (red) on the site of GFP-expressing U87MG and GL261 intracranial gliomas (green).

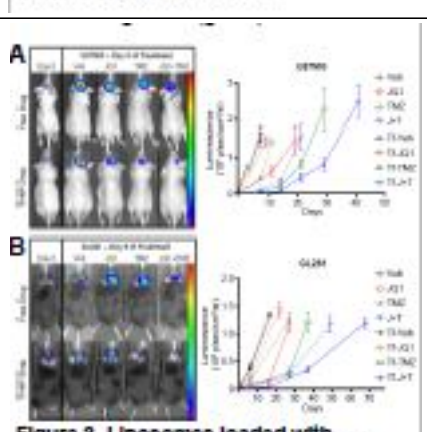

Figure 3. Liposomes loaded with bromodomain inhibitor JQ1 and TMZ have superior pharmacodynamic effects in intracranial orthotopic models of GBM compared to free drug. Bioluminescent images and quantification of average tumor BLI values of A) U87MG and B) GL261 mice taken on day 0 and day 5 following treatment with free drug or drug-loaded Tf-NPs.

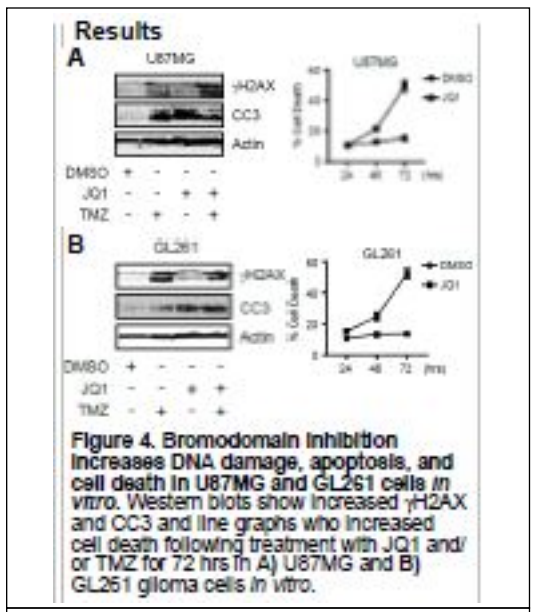

Figure 4. Bromodomain inhibition increases DNA damage, apoptosis, and cell death in U87MG and GL261 cells *in vitro*. Western blots show increased γH2AX and CC3 and line graphs who increased cell death following treatment with JQ1 and/or TMZ for 72 hrs (n A) U87MG and B) GL261 glioma cells *in vitro*.

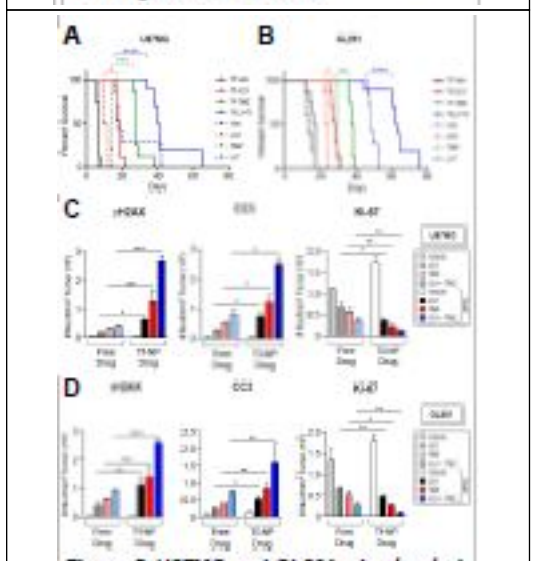

Figure 5. U87MG and GL261 mice treated with JQ1 and TMZ loaded Tf-NPs have additive prolonged survival and demonstrate increased DNA damage and apoptosis in tumors. Kaplan Meier survival plots for: A) U87MG B) GL261 mice. Solid colored lines represent liposome arms. Dotted lines represent free drug arms. Quantification of number of tumor cells that stained positive for markers of DNA damage (γH2AX), apoptosis (CC3), and proliferation (Ki67) in C) U87MG and D) GL261 mice that have received free drug vs liposome-loaded drug (Tf-NP).

Conclusions

Our tumor-targeting nanoparticle offers the potential to deliver novel combination therapies across the BBB to overcome rapid rewiring of resistance pathways as well as avert effects of systemic drug toxicity to improve treatment outcomes for patients with GBM.

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Identify limiting factors to effective delivery of therapies to CNS tumors; 2) Discuss the use of emerging targeted nanotechnologies to improve delivery of novel therapies across the BBB for the treatment of GBM; and 3) Identify novel combination therapies using current FDA-approved drugs and preclinical small molecule inhibitors that that can potentially be used to overcome treatment resistance for GBM.

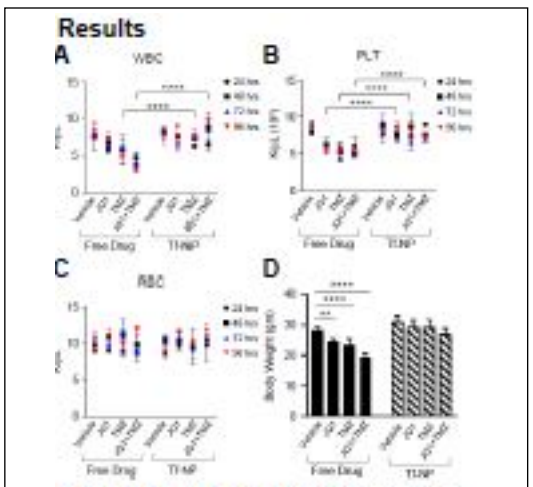

Figure 6. Immunocompetent GL261 mcie treated with Stealth liposomes demonstrate relative protection from the systemically toxic effects of JQ1 and TMZ. Mice treated with drug-loaded Tf-NPs demonstrate relative protection from A) leukopenia and B) thrombocytopenia caused by JQ1 and TMZ. C) Mice in either treatment did not demonstrate drops in RBC counts. D) Quantification of average daily body weights at the end of the 96 hr treatment course.