An *In-vitro* Evaluation of Transferrin-Targeted Resveratrol-Loaded Liposomes for Glioblastoma Treatment

Aditi Jhaveri and Vladimir Torchilin Ph.D., D.Sc.

Center for Pharmaceutical Biotechnology and Nanomedicine (CPBN), Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, 02115, USA

jhaveri.ad@husky.neu.edu

**Purpose:** Glioblastomas (GBM) harbor a sub-population of slow-dividing cells known as tumor-initiating cells (TIC) that are responsible for the formation, maintenance, invasiveness and recurrence of GBM. Conventional chemotherapeutics result in toxicities on normal tissues and act mainly on the tumor bulk while sparing the TICs, which results in tumor relapse. Resveratrol (RES), a natural polyphenol, has been shown to exhibit chemopreventive effects in all the major stages of cancer including initiation, promotion and progression\(^1\). However, RES as a free drug has several limitations including a poor water solubility, chemical instability, low bioavailability, and poor pharmacokinetics\(^2\). To counter these drawbacks, we have developed liposome encapsulated RES (RES-L) and aimed to evaluate its effects on both, the bulk of cancer cells as well as TIC in GBM. RES-L were then modified with transferrin (Tf) as a targeting ligand (Tf-RES-L) to take advantage of the over-expressed Tf receptors (TfRs) in gliomas, and to further enhance the specificity of the formulations.

**Methods:** TIC models were established for two GBM cell lines (U-87 MG and LN-18) using the neurosphere (NS) assay. Formulations were tested for their association with and internalization into cells using flow cytometry and confocal microscopy, respectively. Cytotoxicity assays, cell-cycle analysis, and other flow cytometry assays were carried out to assess the general mechanisms of action of RES on GBM cells.

**Results:** Cytotoxicity studies showed significant time and dose-dependent toxicity of RES-formulations on GBM cells. Tf-RES-L resulted in significantly increased cytotoxicity compared to non-targeted RES-L. RES also inhibited the anchorage-independent growth of GBM NS in a dose-dependent manner. Rhodamine-labeled Tf-L showed a significantly higher association with and internalization into U-87 MG cells versus non-targeted liposomes. RES arrested GBM cells in the S phase of the cell cycle, blocking their S to G2/M progression and also induced significant oxidative stress in GBM cells.

**Conclusions:** From our preliminary studies, liposomal RES seems to be a promising agent for further development against GBM. Targeting TfR significantly enhances the effect of RES-L over the untargeted counterparts.

**References:**
2) Neves, A.R., Lucio, M., Lima, J.L., Reis, S. Medicinal Chemistry; 2012, 19: 1663-1681,