Regression of Glioma Tumor Growth in a F98 Rat Glioma Model by the Nitrone, OKN-007

Rheal A. Towner[1], David L. Gillespie[2], Debra Saunders[1], Osama Abdullah[3], Edward W. Hsu[4], Andrea Schwager[5], and Randy L. Jensen[2]

[1]*Advanced Magnetic Resonance Center, Oklahoma Medical Research Foundation, Oklahoma City, OK, United States,* [2]*Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, United States,* [3]*Bioengineering, University of Utah, Salt Lake City, UT,* [4]*Bioengineering, University of Utah, Salt Lake City, UT, United States,* [5]*Interdepartmental Program in Neuroscience, University of Utah, Salt Lake City, UT, United States*

Synopsis: MR and bioluminescence imaging methods were used to monitor tumor growth, MR spectroscopy was used to assess the effect of OKN-007 on tumor metabolism, and immunohistochemistry (IHC) was used to monitor levels of tumor markers for angiogenesis, cell differentiation or proliferation, and apoptosis, in OKN-007-treated and untreated F98 glioma-bearing rats.

Introduction: Glioblastoma multiforme, a grade IV glioma, has a poor prognosis in humans despite current treatment options. Grading and identification criteria that can be used to provide information regarding tumor behavior include cell proliferation, nuclear atypia, neovascularization and the presence of necrosis and/or apoptotic regions. Many of these grading and identification criteria can be measured with the use of magnetic resonance imaging (MRI) and MR spectroscopy (MRS) methods.

Methods: F98 cells were infected with a modified pMMP retrovirus fused with the coding sequences for luciferase and hygromycin (pMMP-LucHygro; obtained from Dr. S. Lessnick, Univ. of Utah, Huntsman Cancer Institute), and implanted as previously described [1]. OKN007 (Ryss laboratories, Union City, CA, USA) was administered in drinking water (0.018%; ~10 mg/kg body weight/day), starting from 15 days after F98 glioma cell implantations. Rats were anesthetized (isoflurane) and injected i.p. with 50 mg/kg firefly D-Luciferin (Xenogen Corp., Alameda, CA) and photographed 5 min after injection with an IVIS 100 imaging system (Xenogen). Images were quantified using LivingImage software (Xenogen). MRI experiments were done on a 7.0 Tesla/30 cm imaging system (Bruker Biospin, Ettlingen, Germany). Anesthetized animals were placed in a 72 mm quadrature volume coil (transmission), and a surface coil (reception). Rat T_2-weighted imaging was acquired by using RARE with a TR of 3000 ms, a TE of 63 ms, 20 transverse 1 mm-thick slices, a FOV of 3.5×3.5 cm^2, and an in-plane resolution of 137x137 μm^2. MR spectroscopy was acquired using STEAM with a TE of 4.4 ms, a TM (mixing time) of 10 ms, a TR of 3000 ms, 256 averages, and a spectral width of 4006 Hz. Water was suppressed with a VAPOR suppression scheme. LCModel was used for spectral analysis. IHC was used to observe levels of hypoxia inducible factor 1α (HIF-1α), vascular endothelial growth factor (VEGF), carbonic anhydrase IX (CA-IX), glucose transporter 1 (Glut-1), MIB-1 (proliferation index), microvessel density (MVD), and apoptosis (cleaved caspase 3). Statistical differences were obtained using an unpaired, two-tailed Student *t* test (InStat; GraphPad Software, San Diego, CA, USA). A *p* value of less than 0.05 was considered to indicate statistical significance.

Results: From MR and bioluminescence image detection of F98 gliomas, OKN-007 was found to decrease tumor growth ($p<0.05$) (Fig. 1). MR spectroscopy analysis (Fig. 2), indicated F98 glioma-induced alterations in tumor metabolites (tCho (total choline), tCr, NAA (N-acetyl aspartate), Lip1.3 (methylene hydrogens in lipid acyl groups), Lip5.3 (olefinic hydrogens in unsaturated lipid acyl groups)), were found to revert back to normal levels following OKN-007 treatment. IHC was used to assess levels of angiogenic (VEGF, HIF-1α, MVD), cell differentiation (CAIX), cell proliferation (Glut-1, MIB-1), and apoptosis (cleaved caspase 3), markers. OKN-007 resulted in significant decreases in angiogenesis (HIF-1α ($p<0.05$) and MVD ($p<0.05$), but not VEGF) and cell proliferation (Glut-1 ($p<0.05$) and MIB-1 ($p<0.01$)), and a significant increase in apoptosis (cleaved caspase 3 ($p<0.001$)), compared to untreated animals.

Conclusions: MRI and MRS methods were successfully used to monitor the anti-glioma therapeutic effect of OKN-007. Due to its multiple targets (cell proliferation, angiogenesis (although not necessarily through the VEGF pathway), and apoptosis), OKN-007 may be an ideal therapeutic alternative, or may compliment current therapeutic protocols for gliomas.

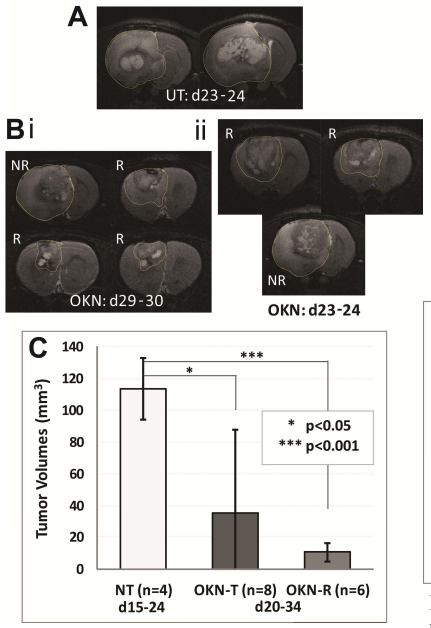

Figure 1: T_2-w MR images of (A) untreated (UT) (n=4), and (B) OKN-007 (OKN)-treated F98 glioma-bearing rats (total and responsive) (n=8). (C) Average tumor volumes (significant decrease in OKN-treated rats (*$p<0.05$ for the total, and ***$p<0.001$ for responsive (n=6) compared to UT animals).

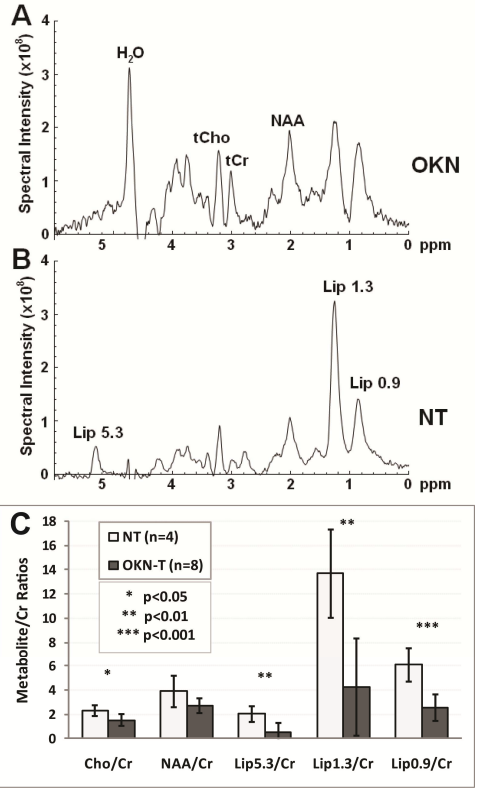

Figure 2: MR spectra from (A) OKN-treated and (B) untreated (UT) rats. Assignments are H_20, tCho, tCr, NAA, and lipids (Lip5.3, Lip1.3, and Lip0.9). (C) Average metabolite/Cr ratios in untreated (UT) and OKN-treated rats. Significant decreases in Cho/Cr (*$p<0.05$), Lip5.3/Cr (**$p<0.01$), Lip1.3/Cr (**$p<0.01$) and Lip0.9/Cr (***$p<0.001$) were observed in OKN-treated rats (n=8) compared to UT animals (n=4).

References:

1. Towner RA, Smith N, Doblas S, Garteiser P, Watanabe Y, He T, Saunders D, Herlea O, Silasi-Mansat R, Lupu F. (2010) *In vivo* detection of inducible nitric oxide synthase in rodent gliomas. *Free Radic Biol Med* 48(5): 691-703.