OKN-007 as an Agent to Assist the Delivery of Anti-Cancer Drugs through the Blood-Brain Barrier

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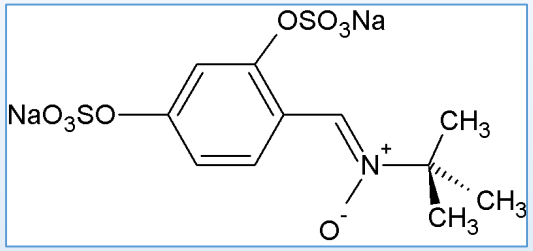
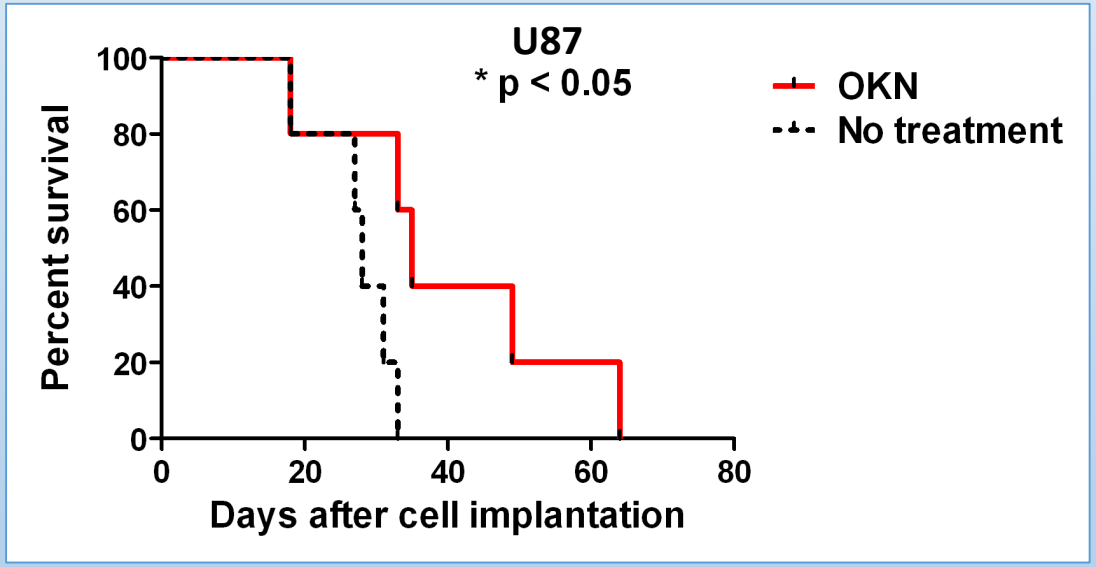
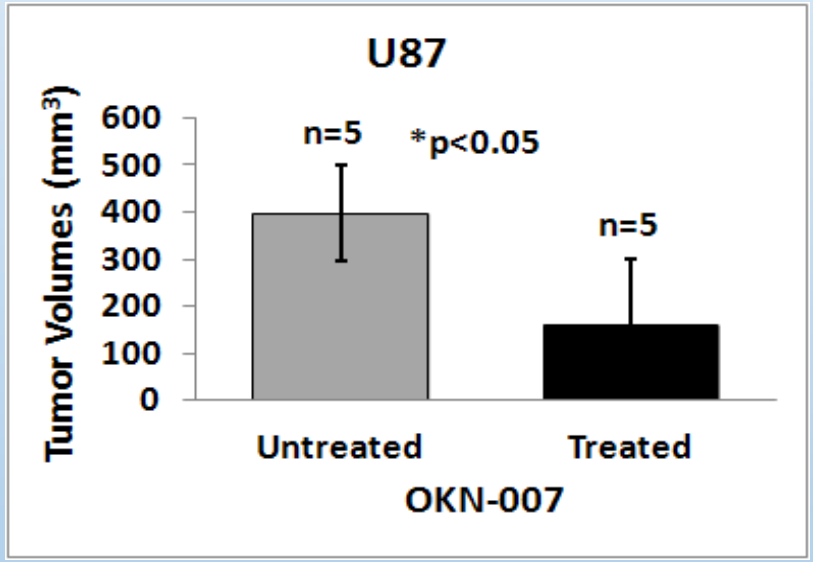
What is OKN-007?



- Small molecule
- Crosses blood-brain-barrier
- Found to significantly decrease tumor volumes and significantly increase survival in several rodent glioma models (mouse GL261, rat C6, F98, human xenograft U87, human pediatric 3752GBM PDX)





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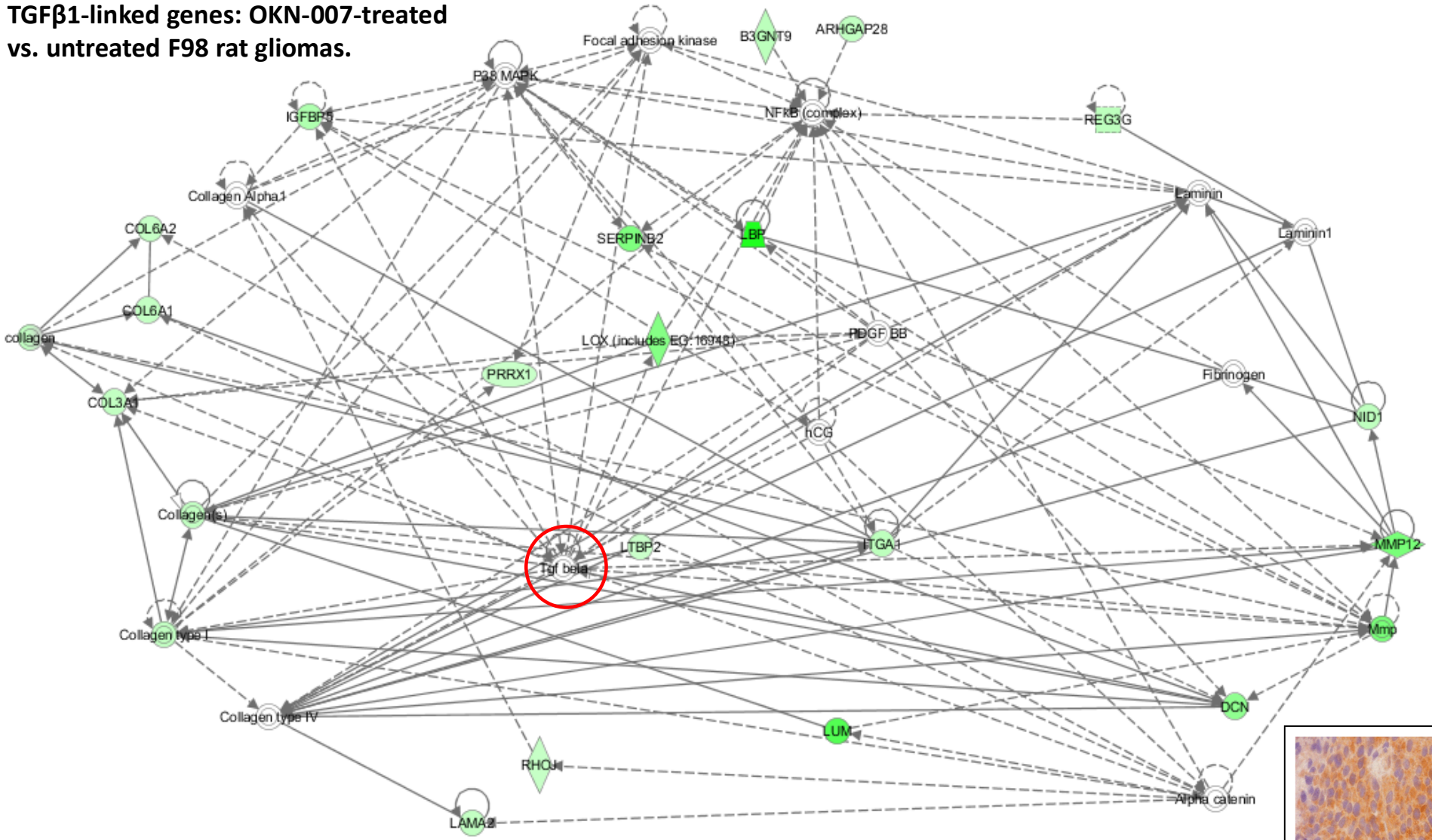
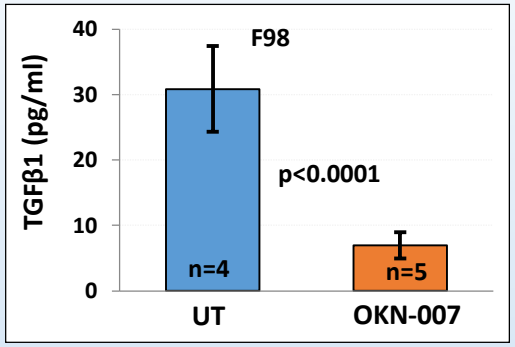
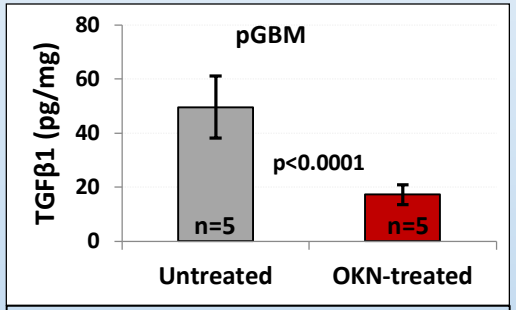
Mechanism(s) of Action – OKN-007

TGFβ1-linked genes: OKN-007-treated vs. untreated F98 rat gliomas.

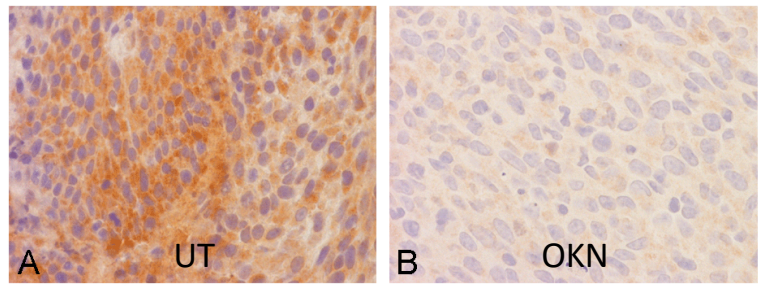






TGFβ1 ELISA. Untreated rat F98 or pGBM gliomas vs. OKN-007-treated gliomas.

TGFβ1 IHC. Untreated (A) and OKN-007-treated (B) rat F98 gliomas.



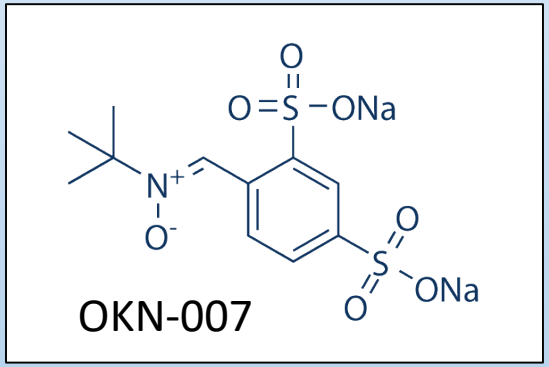
Micro-array data: OKN-007 down-regulates 57 genes including collagens, MMP12 (tissue remodeling), SERPINB2 (serpin peptidase inhibitor), IGFBP5 (insulin-like growth factor binding protein)

OKN-007 and Blood-Brain Barrier (BBB) Permeability

Are there genes associated with OKN-007 and BBB?

Gene	Description	Up-regulation	Down-regulation
ADIPOR2	Adiponectin receptor 2		-2.11
CACNG5	Calcium channel, voltage dependent, gamma subunit 5	2.43	
CCK	Cholecystokinin	2.49	
GFAP	Glial fibrillary acidic protein		-2.48
IGFBP6	Insulin-like growth factor binding protein 6	2.39	
MOG	Myelin oligodendrocyte glycoprotein		-2.16
PDK4	Pyruvate dehydrogenase kinase, isozyme 4		-2.20
S100A1	S100 calcium binding protein A1		-2.87
S1PR5	Sphingosine-1-phosphate receptor 5		-2.14
TP53	Tumor protein p53	2.27	
TTR	Transthyretin (transporter)		-156.81

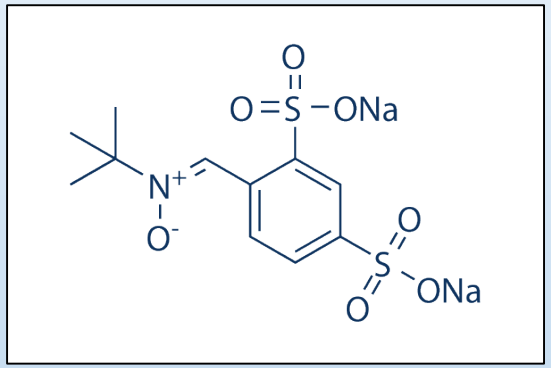
Table 1: Genes associated with OKN-007-induced BBB permeability. Microarray data from normal rat brains, OKN-treated vs. untreated.



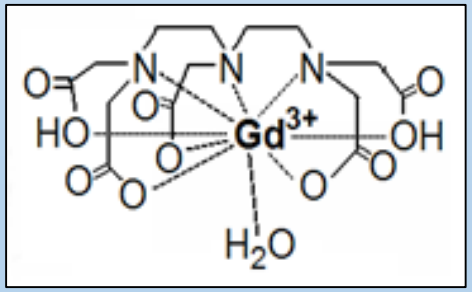
Bioinformatics assessment of genes with shared relationships with BBB from microarray data obtained from normal rat brain, either treated with OKN or untreated, indicated that there were at least 3 genes (one associated with a calcium channel) that were up-regulated >2-fold, and at least 7 genes (one associated with a calcium binding protein; and another, transthyretin (>150-fold) a transporter associated with receptor-mediated transcytosis) that were down-regulated >2-fold by OKN.

Can OKN-007 be used to allow entry of the MRI contrast agent, Gd-DTPA, through the BBB?

- MRI contrast agent, Gd-DTPA, does not cross BBB of normal brain.
- If Gd-DTPA crosses BBB, then MRI can be used to detect an increased signal intensity in brain tissue *in vivo*, due to presence of the contrast agent.
- Initially determine if Gd-DTPA can cross BBB in presence of OKN-007 in mice (both administered i.v.)
- Also establish the timing of how long OKN-007 opened up the BBB, by assessing the presence of Gd-DTPA which increased MRI signal intensities at different time-points. Gd-DTPA was administered at different times following OKN-007.

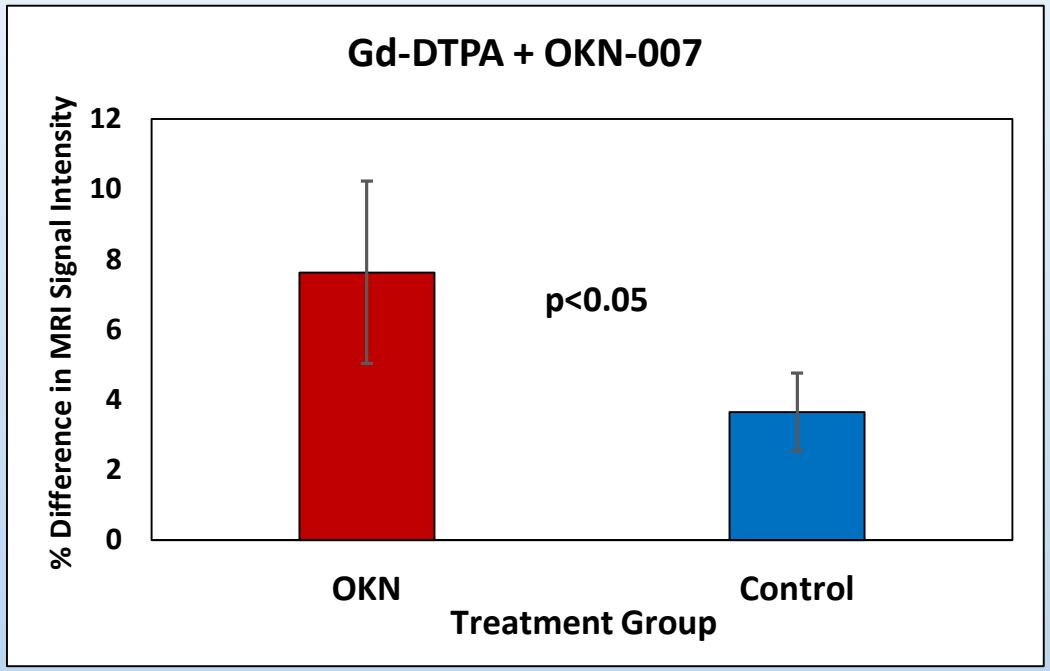


OKN-007: MW 381.33

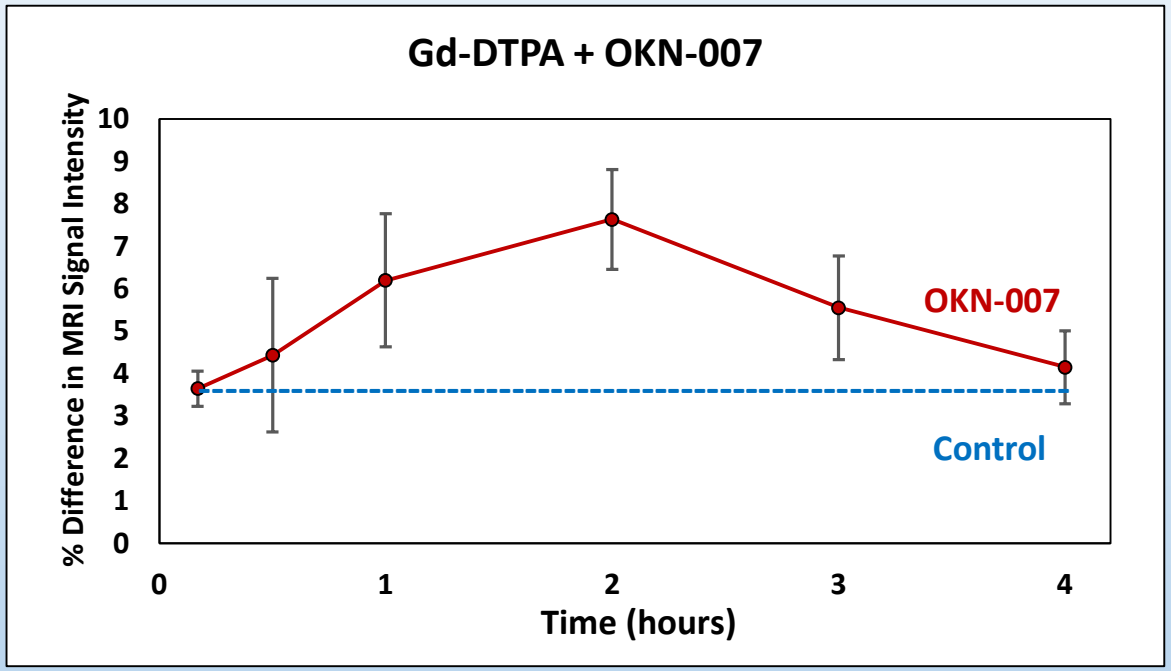


Gd-DTPA: MW 545.56

OKN-007 Increases BBB Permeability Delivery of MRI Contrast agent, Gd-DTPA



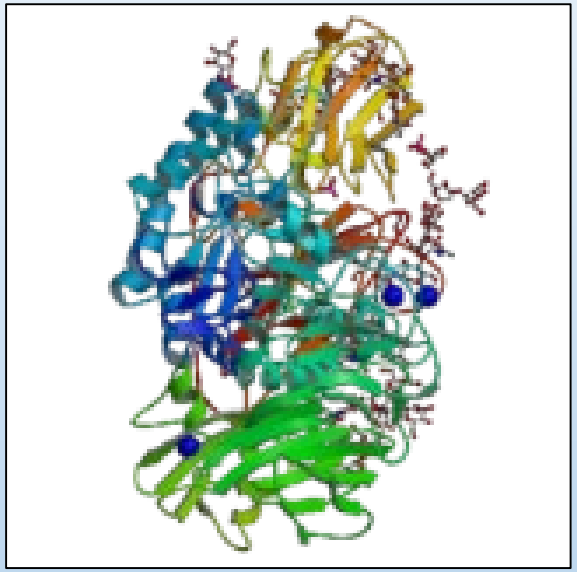
OKN-007 increases BBB permeability to allow MRI contrast agent, Gd-DTPA, into the brain, as depicted by a significant increase in percent (%) difference in MRI signal intensity ($p<0.05$), when compared to a control administered Gd-DTPA without OKN-007.



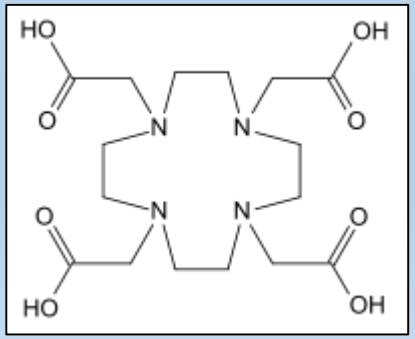
Increase in BBB permeability uptake of MRI contrast agent, Gd-DTPA, by OKN-007, occurs at ~2 hours following i.v. administration of OKN-007 in mouse brains, as depicted by an increase in percent (%) difference in MRI signal intensity. Control value is included for comparison.

Can OKN-007 be used to allow entry of a large protein, such as β-Galactosidase, through the BBB?

- B-Galactosidase has a MW of 465 kDa, and can't cross the BBB in normal brain.
- Attached MRI contrast agent, Gd-DOTA, to β-galactosidase via a NHS-link to cysteine residues.
- Compared mice that were administered Gd-DOTA-β-galactosidase with OKN-007, or without OKN-007. Gd-DOTA-β-Galactosidase was administered one hour after OKN-007. Both were administered i.v. via a tail-vein catheter.

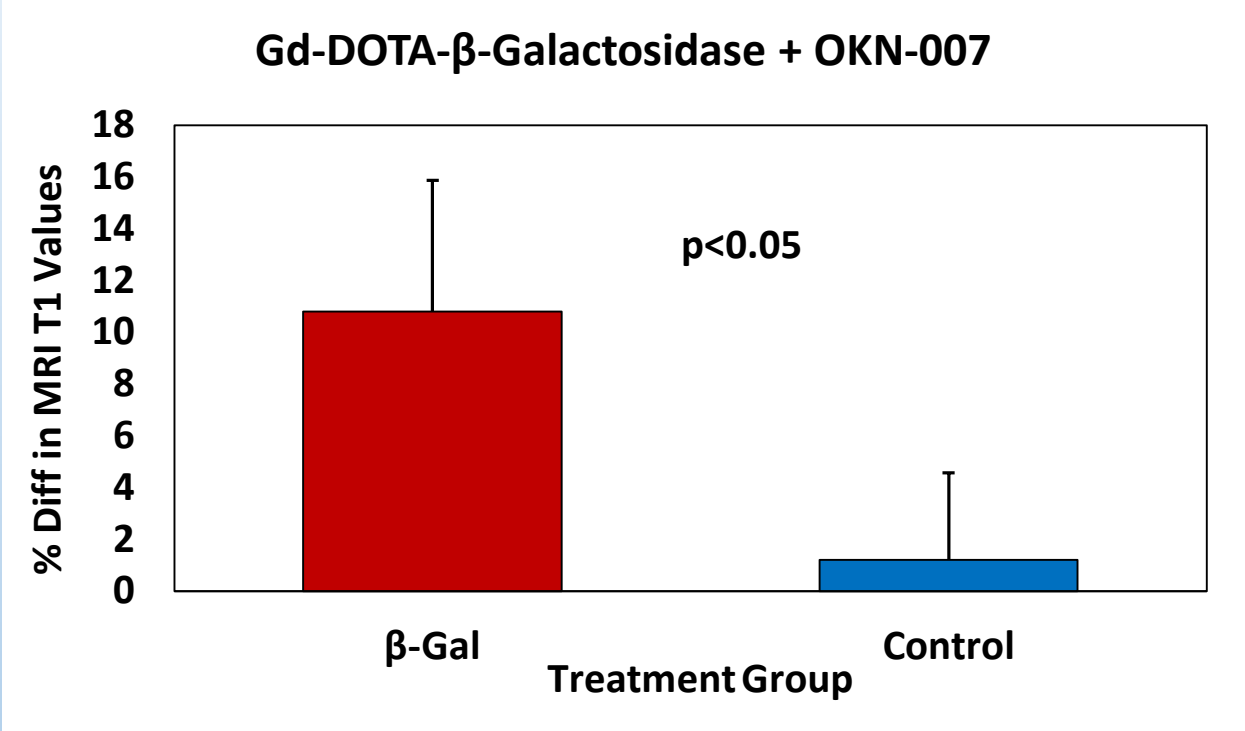


B-Galactosidase



Gd-DOTA: MW 404.42

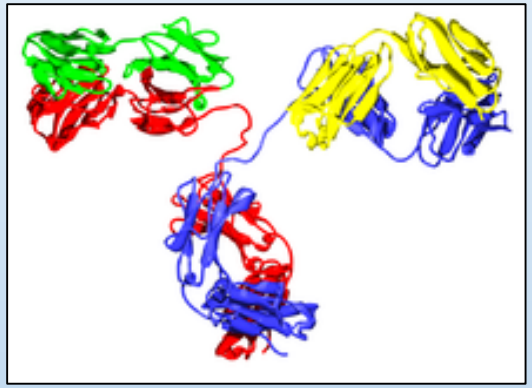
OKN-007 Increases BBB Permeability Delivery of β-Galactosidase



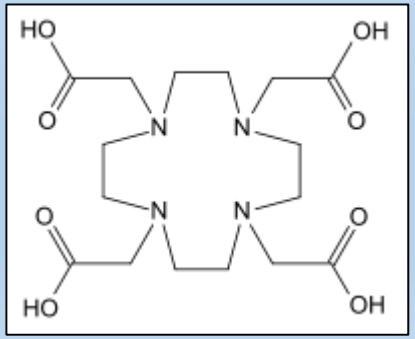
OKN-007 significantly increases BBB permeability of Gd-DOTA-labeled β-Galactosidase (β-Gal) in mouse brains, when compared to a control that was administered Gd-DOTA-β-Gal without OKN-007 ($p<0.05$). BBB changes were assessed by evaluating the MRI parameter, T1 relaxation value. Without OKN-007, Gd-DOTA-β-Gal does not cross the BBB.

Can OKN-007 be used to allow entry of an antibody, such as anti-EphB2, which targets the neuronal biomarker EphB2, through the BBB?

- Antibodies (IgG) (MW of 150 kDa) can't cross BBB in normal brain.
- Attached MRI contrast agent, Gd-DOTA to anti-EphB2 antibody via a NHS-link to cysteine residues.
- Targeted approach was used to determine if an antibody to a neuronal marker could attach.
- Fluorescence imaging could also be used to verify the presence of the antibody in normal brain.
- Our experiment compared mice that were administered Gd-DOTA-anti-EphB2 with OKN-007, or without OKN-007. Gd-DOTA-anti-EphB2 was administered one hour after OKN-007. Both were administered i.v. via a tail-vein catheter.

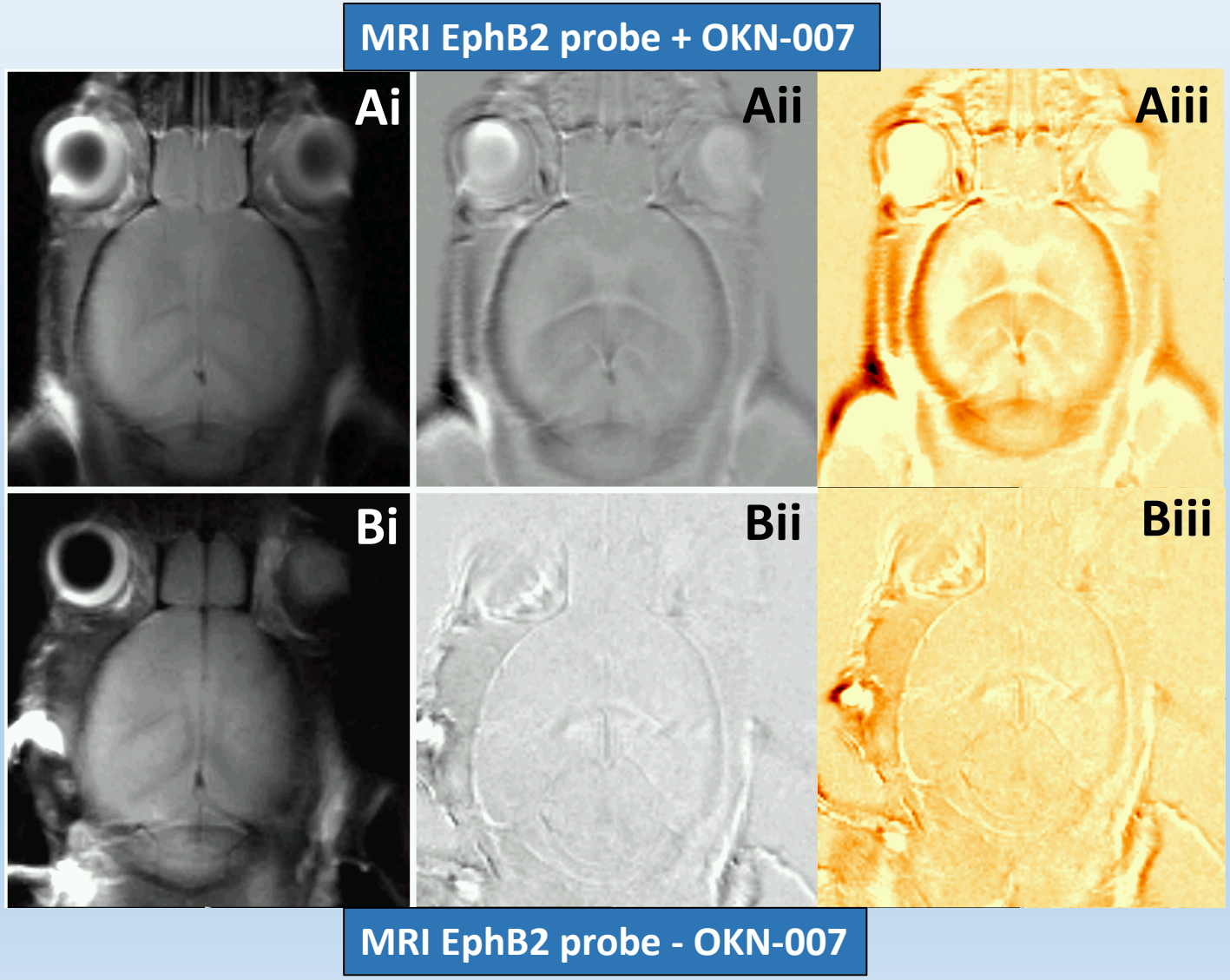


Antibody: MW 150 kDa



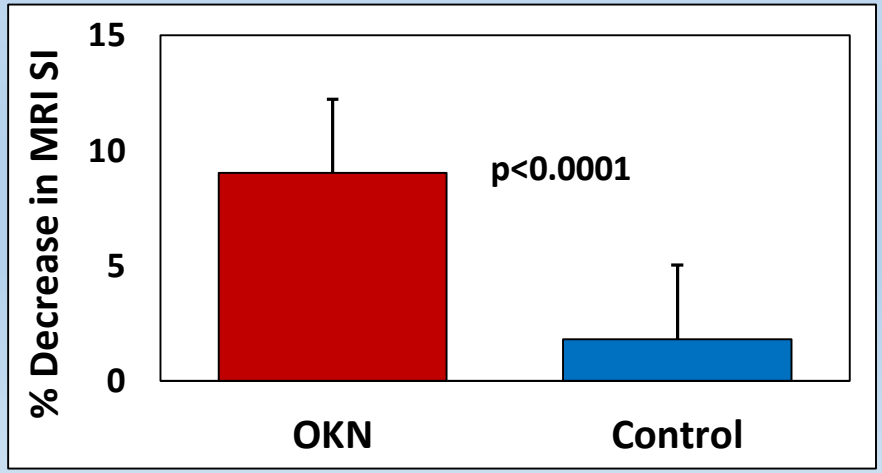
Gd-DOTA: MW 404.42

OKN-007 Allows an Antibody Targeting EphB2 Coupled to a MRI Contrast Agent to Cross the BBB

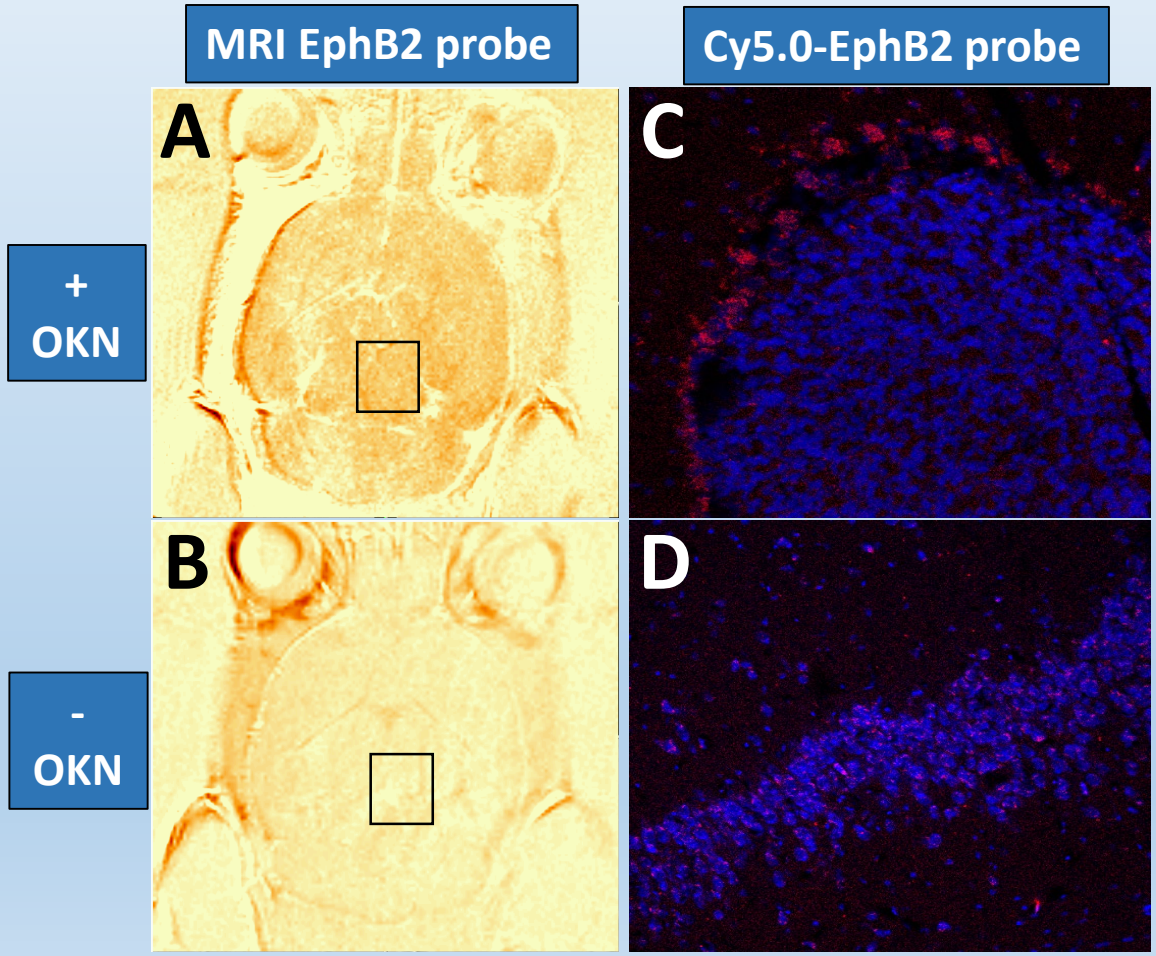


OKN-007 significantly increases MRI signal intensity, indicating uptake through BBB, of Gd-DOTA-labeled anti-EphB2 antibody (EphB2 probe) in mouse brains, when compared to a control that was administered Gd-DOTA-anti-EphB2 without OKN-007 ($p<0.01$) (see panel C). A: OKN-007 + anti-EphB2 probe; B: Anti-EphB2 probe with no OKN-007; i: MR image; ii: Contrast difference image; iii: Colorized contrast difference image. Note darkened region (panels A ii/iii).

C



OKN-007 Allows an Antibody Targeting EphB2 Coupled to a MRI Contrast Agent to Cross the BBB: Confirmation with Fluorescence Microscopy



OKN-007 increased MRI signal intensity of a Gd-DOTA-labeled anti-EphB2 antibody (EphB2 probe) in mouse brains is confirmed with fluorescence probe (Cy5.0-anti-EphB2 antibody). MRI contrast difference images of (A) OKN-007 + anti-EphB2 probe; and (B) anti-EphB2 probe with no OKN-007. Note dark orange area in most brain regions, but particularly in central image region in panel A. Outlined region in panel A depicts region for fluorescence microscopy of Cy5.0-anti-EphB2 probe. Confirmation with fluorescence imaging of Cy5.0-anti-EphB2 antibody in (C) OKN-007 + Cy5.0-anti-EphB2 (red) (corresponding to location outlined in A) ; and (D) Cy5.0-anti-EphB2 with no OKN-007 (corresponding to location outlined in B). 20x magnification. Note that Cy5.0-anti-EphB2 probe is only found in OKN-007-treated brain tissue. Gd-DOTA-anti-EphB2 probe and Cy5.0-anti-EphB2 probe were administered (tail-vein catheter) at the same time in a 50:50 mixture.

Summary – OKN-007 as an agent to open BBB permeability

- OKN-007 is able to open the BBB for a 1-2 hour window
- OKN-007 allows both small (MW ~300) and large molecules (> 450kD) to cross the BBB

Future studies

- Establish if OKN-007 can be used to augment the delivery of currently used or therapeutic agents in clinical trials for metastatic brain tumors (e.g. metastatic breast, colon or liver cancers)
- Establish if OKN-007 can be used to augment the delivery of currently used or therapeutic agents in clinical trials for other neurological disorders