# Applicability of Bioluminescence and MRI for Monitoring Cell-Based Therapy of Demyelination

**P. Walczak[1,2], T. Magnus[3], A. A. Gilad[1,2], D. A. Kedziorek[1,2], M. T. McMahon[4], D. A. Kerr[5], and J. W. Bulte[1,2]**

[1]Department of Radiology, Johns Hopkins University, Baltimore, MD, United States, [2]Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, United States, [3]Gerontology Research Center, Stem Cell Biology Unit/Laboratory of Neuroscience, National Institute on Aging, National Institutes of Health, Baltimore, MD, United States, [4]F.M. Kirby Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States, [5]Department of Neurology, Johns Hopkins University, Baltimore, MD, United States

Introduction: Therapeutic intervention of neurodegenerative disorders remains one of the major challenges of modern medicine. A new approach is the use of stem/progenitor cells to replace dead or defective endogenous cells. Multiple sclerosis and other myelin disorders can benefit from cell-based therapy using myelinating oligodendrocytes. The current major issues in neurotransplantation are the selection of the appropriate cell type, ensuring graft survival, maximizing the optimal cell distribution, and enabling cellular differentiation. Using MRI with its exquisite resolution it is possible to precisely monitor the delivery and distribution of implanted cells but it lacks information about functional graft status. This missing information can be supplemented with bioluminescent imaging (BLI) allowing the assessment of cell survival and phenotypic features of genetically engineered cells. Here we employed two cellular imaging modalities, MRI and BLI, to probe whether their sensitivity is sufficient for the detection of cell numbers that are typically being used in neurotransplantation.

Methods: Mouse glial restricted precursors (GRPs) were transfected with the lentiviral vector EF.luc-IRES.GFP in order to express luciferase and green fluorescent protein (GFP). FACS-sorted cells were expanded and further labeled with the MR contrast agent Feridex. Labeled cells ($2x10^5$/4µl) were stereotaxically implanted into immunodeficient NOD-SCID mice (AP=-1.5, ML=1.0, DV=1.0) The cell graft was placed 3mm away from an ethidium bromide-induced demyelinated lesion, induced three days prior to cell grafting (AP=-1.5, ML=1.0, DV=2.0). BLI imaging was performed 1, 3, 15 and 28 days after cell implantation. For BLI mice, were anesthetized by i.p. injection of Ketamin/Acepromazin (100/10mg/kg), followed by i.p. injection of luciferine (150mg/kg). The skull was exposed (to enhance light penetration) by a 1 cm skin incision, and mice were imaged using an IVIS 200 system (Xenogen). For *in vivo* MRI, the animals were isoflurane-anesthetized and imaged with a Bruker 9.4T spectrometer. In vivo imaging was performed at 1, 3, and 15 days after cell implantation with a T2-weighted spin echo sequence (TE/TR=26/2000ms, AV=2, RES=230x156µm). High-resolution ex vivo MRI of brains removed 1 or 28 days after cell implantation were obtained using a T2*-weighted 3D gradient echo sequence (TE/TR=5/100ms, AV=4, RES=83x83x83). For validation of the imaging results, brain tissue was cryosectioned and stained with Luxol fast blue for demyelination and Prussian blue for detection of Feridex iron. Immunohistochemical detection of transplanted cells was performed using anti-GFP and anti-luciferase antibodies (Chemicon).

Results: Injection of ethidium bromide resulted in extensive demyelination starting 4 days after ethidium bromide injection (A, B). Luciferase-expressing GRPs implanted 1mm deep into the brain generated a strong BLI signal (C) The BLI signal dropped sharply during the first 15 days after transplantation followed by stabilization at about 5% of initial signal (C, graph), indicating that only a minority of cells survive for prolonged times. The GFP (D) and luciferase (E) reporter genes allowed detection of grafted cells in microscopic sections. Cellular MRI demonstrated the precise site of cell injection one day after implantation (white box in F). At this time point, the MRI correlated closely with the histological Prussian Blue (G) and anti-GFP staining (H). However, despite the low survival rate and cell loss, the apperance of hypointense signal did not change over the 28-day period.

Conclusions: Both MRI and BLI are capable of detecting neurotransplanted cells. MR imaging can provide detailed information about the initial cell distribution but cannot report on functional cell status. BLI, at the other hand, has a very low spatial resolution, but allows longitudinal monitoring of cell viability. Combining the two complementary modalities should provide a better insight in the fate of transplanted cells.

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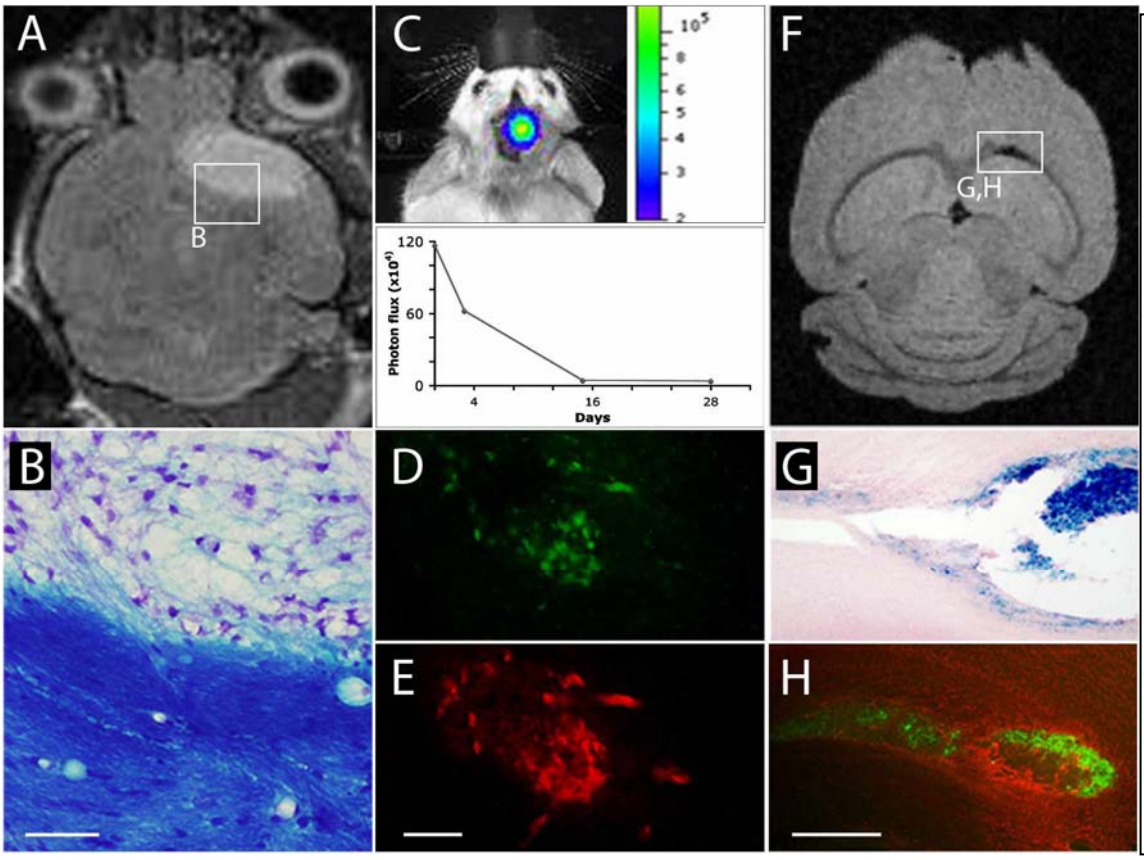


(A) T2-weighted MRI of mouse brain 4 days after ethidium bromide injection reveals a large demyelinated lesion. (B) Luxol fast blue staining of box insert in (A), demonstrating severe demyelination (upper part). (C) Upper panel: BLI 15 days after GRP transplantation, lower panel: quantitative analysis of BLI signal recorded over 28 days after transplantation. (D) Histology 1 day after GRP transplantation with anti-GFP immunostaining. (E) Anti-luciferase immunostaining of the same tissue section as in (D). (F) T2*-weighted MRI of brain tissue 1 day after implantation of magnetically labeled GRPs reveals, hypointense area within the right corpus callosum. (G) Microscopic section corresponding to white box in (F) stained for Prussian Blue. (H) Adjacent section from the same area stained for anti-GFP (green) and myelin basic protein (red).