AJNR

Noninvasive Molecular Neuroimaging Using Reporter Genes: Part II, Experimental, Current, and Future Applications

T.F. Massoud, A. Singh and S.S. Gambhir

AJNR Am J Neuroradiol 2008, 29 (3) 409-418
doi: https://doi.org/10.3174/ajnr.A0863
http://www.ajnr.org/content/29/3/409

REVIEW ARTICLE

Noninvasive Molecular Neuroimaging Using Reporter Genes: Part II, Experimental, Current, and Future Applications

T.F. Massoud
A. Singh
S.S. Gambhir

SUMMARY: In this second article, we review the various strategies and applications that make use of reporter genes for molecular imaging of the brain in living subjects. These approaches are emerging as valuable tools for monitoring gene expression in diverse applications in laboratory animals, including the study of gene-targeted and trafficking cells, gene therapies, transgenic animals, and more complex molecular interactions within the central nervous system. Further development of more sensitive and selective reporters, combined with improvements in detection technology, will consolidate the position of in vivo reporter gene imaging as a versatile technique for greater understanding of intracellular biologic processes and underlying molecular neuropathology and will potentially establish a future role in the clinical management of patients with neurologic diseases.

Molecular imaging seeks to shed new light on both structure and function by creating images that directly or indirectly reflect specific cellular and molecular events (eg, gene expression) that can reveal pathways and mechanisms responsible for disease within the context of physiologically authentic and intact living subject environments.[1] We and others have previously reviewed the factors contributing to the emergence of molecular imaging, the particular advantages of these approaches, and the general goals potentially achievable in biomedical research and clinical practice by adopting molecular imaging strategies.[1-5] One of the subdisciplines in molecular imaging that is least familiar to clinical imaging specialists, and arguably one that holds future promise in neuroimaging, is reporter gene expression imaging.[6,7] In the first article of this series, we reviewed the basic principles and recent technologic developments in reporter gene expression imaging in living subjects.[8] In this second article, we review examples from the myriad experimental applications currently possible in molecular neuroimaging.

Experimental Applications in Molecular Neuroimaging Using Reporter Genes

Four broad categories of experimental applications for reporter gene imaging in the brain are as follows: gene marking of cells and viruses with reporter genes, imaging of gene therapies, imaging of transgenic animals carrying reporter genes, and imaging of more complex intracellular molecular events such as protein trafficking. Some details of recent examples of these applications are displayed in the Tables, with 1 or 2 representative examples of each application discussed in greater detail below. Of note regarding terminology, "xenografts" result from transplantation of cells or tissues from 1 species to another (eg, human cells into mice), and "orthotopic transplants" refer to grafting cells into the same body location/organ as that from which the cells are derived (eg, glioma cells delivered into brain).

From the Department of Radiology (T.F.M., A.S.), Section of Neuroradiology, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, UK; and the Molecular Imaging Program at Stanford (T.F.M., S.S.G.) and the Departments of Radiology and Bioengineering (S.S.G.), Bio-X Program, Stanford University School of Medicine, Stanford, Calif.

Please address correspondence to Tarik F. Massoud, MD, PhD, Department of Radiology, Section of Neuroradiology, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Box 219, Level 5, Hills Rd, Cambridge CB2 2QQ, United Kingdom; e-mail: tfm23@radiol.cam.ac.uk

DOI 10.3174/ajnr.A0863

Imaging Gene-Marked Cells

Gene marking may be used to track the behavior of almost any tissue.[9] It is necessary to transfect cells stably with the imaging marker gene if they and their progeny are to be followed for their entire lifespan within the living subject. However, this assumes that minimal or no promoter attenuation or shutoff takes place. The latter can contribute substantially to a decline in transgene expression despite the constitutive nature of the promoter. In practice, transient transfection of cells suffices if these marked cells are to be imaged in a living subject for no more than approximately 7–10 days, depending on the cells in question and other parameters as well.[1] In principle, gene-marker studies may be used to follow the behavior of almost any cell type in living subjects. In clinical practice, this has been mostly used with hematopoietic cells.[9] However, in molecular imaging research, a variety of cells can be engineered to incorporate reporter genes. Usually, gene marking of cells that are static in 1 location (eg, subcutaneous tumor xenografts or orthotopic brain tumor implants) is used for first assessment and continued validation of reporter genes and their probes, for refining the technical aspects of molecular imaging signal-intensity detection from the brain, or for studying the behavior of the cells themselves within living subjects. This can be accomplished in 2 ways: ex vivo transfection of the cells in question with a vector containing an imaging cassette, followed by placement of these cells in a living subject or direct in vivo placement, usually via injection of the vectors carrying the reporter gene, as part of the recombinant genome of viruses, into the cells of interest within the body.

There are numerous examples of bioluminescence imaging of cells (especially cancer cells) that are mostly destined to remain static in the brain after ex vivo gene marking with imaging reporters and subsequent placement in living rodents (Table 1).[10-23] A noteworthy advantage in these cancer models is that they create the opportunity for temporal evaluation of cancer biology in a noninvasive manner. Dynamic studies of xenograft growth and regression, either spontaneously or after therapy, can be performed. The enzymatic emission of light by *firefly* luciferase (Fluc) is adenosine triphosphate–dependent; therefore, only living metabolically active cells contribute to

Table 1: Recent examples of applications in molecular neuroimaging using gene marking (of static cells)

Type of Cell Marked	Method of Gene Marking	Transplant Site	Animal Model	Imaging Method	Application	Reference
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of technical aspects of neuroimaging by testing suitability of Fluc reporter for brain imaging; evaluation of antineoplastic chemotherapy	10
Cancer cells	Ex vivo	Orthotopic	Mice, rats	Fluc BLI	Evaluation of technical aspects of neuroimaging after lentiviral transduction of various cancer cells	11
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of technical aspects of neuroimaging using a herpes simplex virus amplicon vector expressing Fluc from an inducible promoter	12
Cancer cells	Ex vivo	Orthotopic, heterotopic	Mice	Fluc BLI	Evaluation of technical aspects of neuroimaging by comparing level and time course of light signal from 2 different locations	13
Cancer cells	Ex vivo	Orthotopic, heterotopic	Mice	HSV1-tk, PET	Evaluation of technical aspects of neuroimaging by testing suitability of a ^{76}Br-labeled uracil analog as a probe in brain imaging	14
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of technical aspects of neuroimaging by correlating tumor growth with Fluc BLI and MR imaging; evaluation of antineoplastic chemotherapy	15
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of technical aspects of neuroimaging when establishing tumors with varying abilities to disrupt the BBB; evaluation of antineoplastic chemotherapy	16
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of technical aspects of neuroimaging by testing hyperspectral/multispectral light analysis as a means of 3D localization in BLI	17
Normal brain	In vivo	Orthotopic	Mice	GFP, fluorescence	Evaluation of technical aspects of neuroimaging using reflectance fluorescence imaging	18
Normal brain	In vivo	Orthotopic	Rats	HSV1-tk, PET	Evaluation of technical aspects of neuroimaging in diagnosing early herpes simplex encephalitis	19
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of role of activation of G protein-coupled receptor CXCR4 in growth of intracranial tumors; evaluation of antineoplastic chemotherapy	20
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of immunotherapy of intracranial tumors	21
Cancer cells	Ex vivo	Orthotopic	Rats	Fluc BLI	Evaluation of photodynamic therapy of intracranial tumors	22
Cancer cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of tumor angiogenesis by imaging integrin $\alpha_v\beta_3$ receptor expression using fluorescence imaging	23

Note:—BLI indicates bioluminescence imaging; GFP, green fluorescent protein; PET, positron-emission tomography; HSV1-tk, herpes simplex virus–thymidine kinase; BBB, blood-brain barrier; $\alpha_v\beta_3$, a vitronectin receptor on the cell surface.

the signal intensity. A decrease in signal intensity occurs as cells die.

One of the earliest applications of reporter gene imaging of orthotopic mouse brain xenografts of rat 9L gliosarcoma cells gene-marked with *Fluc* was conducted by Rehemtulla et al.[10] Intracerebral tumor burden was monitored over time by quantification of light emission and tumor volume by using bioluminescence imaging and MR imaging, respectively. There was excellent correlation ($r = 0.91$) between detected photons and tumor volume. A quantitative comparison of tumor cell kill, determined from serial MR imaging volume measurements and bioluminescence imaging photon counts following 1,3-bis(2-chloroethyl)-1-nitrosourea treatment, revealed that both imaging techniques yielded statistically similar cell kill values ($P = .951$). These results provide direct validation of bioluminescence imaging as a powerful and quantitative tool for the assessment of antineoplastic therapies in living animals.

In a more recent study, Deroose et al[11] reported the use of bioluminescence imaging to characterize lentiviral vector–mediated gene transfer into mouse brain. Various features of the imaging signals were characterized including localization (Fig 1), kinetics, resolution, and reproducibility. Although light signal intensity gradually decreased to 20% of initial val-

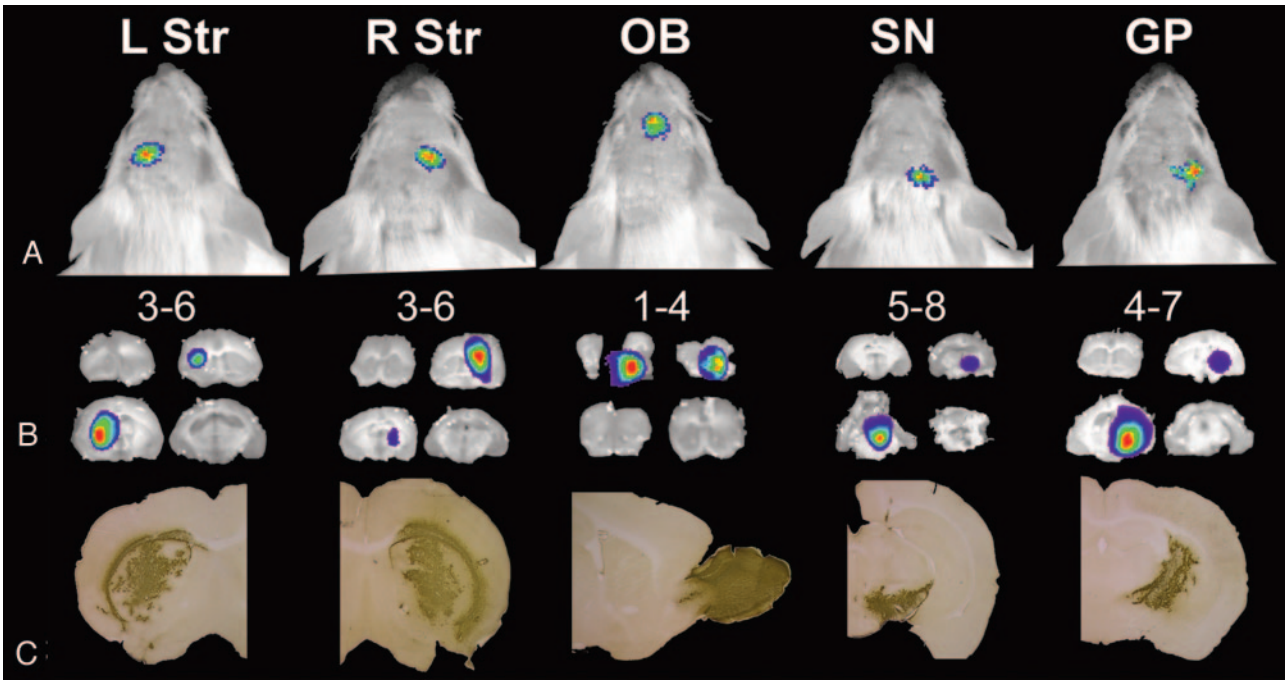

Fig 1. The localization of the bioluminescence imaging signal intensity reflects the anatomic site of injection. *A*, Bioluminescence imaging scans 14 days after injection of 293T cells transduced with a lentiviral construct encoding enhanced green fluorescent protein (EGFP) and Fluc separated by the internal ribosome entry site (I) of the encephalomyocarditis virus (LV-EGFP-I-Fluc) showing a focus that is located above the injection site: caudal to the eyes and on the left side of the head for the left striatum (L Str), caudal to the eyes and on the right side for the right striatum (R Str), between the eyes for the olfactory bulb (OB), near the caudal edge of the skull for the substantia nigra (SN), and intermediate between R Str and SN for the globus pallidus (GP). These sites correspond to the expected locations on the basis of the injection coordinates. *B*, Ex vivo bioluminescence images of 1-mm-thick coronal sections show the localization of the signal intensity at the site of injection. The sections are numbered in the anteroposterior direction from the bulbus olfactorius[1] to the cerebellum.[8] *C*, Immunohistochemistry for EGFP confirms the site of injection. (Reprinted by permission from Macmillan Publishers: Deroose et al. *Mol. Therapy* 2006;14:423–31, copyright 2006).

ues obtained in the first month, the signal intensity remained constant thereafter for more than 1 year after heterotopic brain xenografting of stably transduced 293T cells (Fig 2), allowing the potential for long-term evaluation of novel therapies for experimental brain disorders.

In vivo imaging of cell trafficking is currently performed in clinical practice (eg, by using indium-111 oxine for single-photon emission tomography [SPECT] imaging of infection and inflammation[24]) and is the objective of many immunologic and oncologic studies. Gene marking has the advantage over simple cell labeling for long-term tracking of cells because the imaging gene is passed on to the cell progeny and the imaging signal intensity is not lost through dilution by egress of the label from the cell.[25] When a gene-marked cell dies or is phagocytosed by immune cells, the imaging signal intensity is also lost, unlike the situation with simple cell labeling in which the imaging signal intensity is not dependent on cell viability and may originate from extracellular space or from within immune scavenger cells.[25]

Table 2 outlines examples of recent reports that feature reporter gene imaging of trafficking virus particles,[26-28] parasites,[29] cancer cells,[30] and stem cells to the brain.[31-35] Viral marking is used mostly to study the pathogenesis of viral encephalitis, whereas marking of cancer cells may be used to investigate brain metastases from systemic primary sources (eg, breast cancer). An application gaining rapid acceptance in the laboratory is that of reporter gene imaging of neural stem cells to visualize, quantify, and time their trafficking to gliomas,[31,32] ischemic brain,[33,34] and injured spinal cord.[35] Kim et al[33] noninvasively imaged the migratory behavior of *Fluc*-marked neural progenitor cells to middle cerebral artery in-

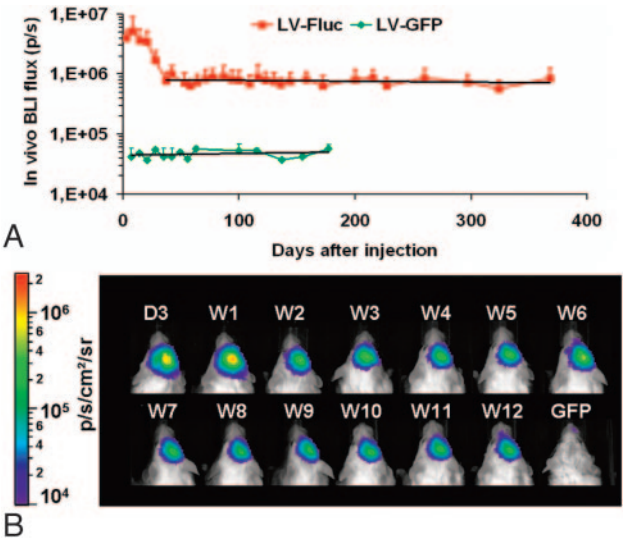

Fig 2. Time course of bioluminescence imaging (BLI) signal intensity after lentiviral (LV) transduction of mouse brain. *A*, Long-term evolution of the BLI signal intensity in a group of mice ($n = 10$) injected with 17-ng p24 of LV-Fluc and in a group injected with 8.4-ng p24 control vector (LV-enhanced green fluorescent protein [EGFP], $n = 4$). After a peak at days 8 to 14, the signal intensity declines during the first month to 16% of the maximum value at day 37 and then remains constant at 17.5 ± 2.3% of the maximum value from days 42 to 365 ($R^2 = 0.027$) for LV-Fluc and for all time points for LV-EGFP ($R^2 = 0.041$). *B*, BLI of a representative animal shows an initial rise in signal intensity at week (W) 1 followed by a decrease and thereafter a stabilization of the signal intensity. The control animal shown represents the highest signal intensity seen in a control animal. GFP indicates green fluorescent protein; D, day; p, photons. (Reprinted by permission from Macmillan Publishers: Deroose et al. *Mol. Therapy* 2006;14:423–31, copyright 2006)

farcts in mice and found that intraventricular delivery of stem cells results in earlier and more efficient infarct seeding. More

Table 2: Recent examples of applications in molecular neuroimaging using gene marking (of trafficking cells)

Type of Cell Marked	Method of Gene Marking	Transplant Site	Animal Model	Imaging Method	Application	Reference
Viruses	Ex vivo	Intravenous, or intranasal	Mice	Fluc BLI	Evaluation of effects of interferons on vaccinia viral spread to the brain	26
Viruses	Ex vivo	Intravenous	Mice	Fluc BLI	Evaluation of factors relating to Sindbis viral spread to the brain	27
Viruses	Ex vivo	Intravenous	Mice	Fluc BLI, Rluc BLI	Evaluation of effects of valacyclovir on HSV-1 viral spread to the brain and eyes	28
Malaria parasites in RBCs	Ex vivo	Intravenous	Mice, rats	Fluc BLI	Evaluation of biology of parasite sequestration in cerebral malaria	29
Cancer cells	Ex vivo	Intravenous	Mice	Fluc BLI	Evaluation of breast cancer metastasis to brain	30
Stem cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of trafficking of stem cells to brain tumors	31
Stem cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of trafficking of stem cells to brain tumors	32
				Fluc BLI	Evaluation of effect of stem cell-delivered chemotherapy on tumor burden	
Stem cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of trafficking of stem cells to brain infarcts	33
Stem cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of trafficking of stem cells to ischemic brain in relation to immune status and host immunity	34
Stem cells	Ex vivo	Orthotopic	Mice	Fluc BLI	Evaluation of trafficking of stem cells to injured spinal cord	35

Note:—BLI indicates bioluminescence imaging; RBC, red blood cells; Rluc, *Renilla* luciferase.

recently, the same group showed that marked stem cells survived better in T-cell–deficient nude mice than in immunocompetent animals, indicating that immune status and host immunity can have an influence on stem cell graft survival in the cell therapy of experimental stroke.[34]

Imaging of Gene Therapies

Although various methods of gene therapy have met with limited success, it is probable that eventually many diseases will be successfully treated with the delivery of 1 or more transgenes to target tissue. A concern in applying gene therapy is achievement of controlled and effective delivery of genes to target cells and avoidance of ectopic expression. Molecular imaging of reporters on particular therapeutic genes could be critical in optimizing gene therapy.[36] The aim of these approaches is to image quantitatively reporter gene expression and from this to infer levels, location, and duration of therapeutic gene expression. There are several molecular strategies to achieve linkage of expression of the therapeutic transgene and the imaging reporter gene.[37] These various techniques can be adopted with bioluminescence imaging reporter genes.

To date, several gene therapy studies have incorporated reporter gene imaging either to image the trafficking of the therapeutic transgene delivery vehicle alone,[38] to image the target tissue in the brain alone (eg, to image the effect of therapy on an intracranial glioma by gene marking the glioma cells themselves),[39,40] or when expression of the imaging gene is linked with that of the therapeutic gene to quantify transgene expression in the brain (Table 3).[41] As an example of the latter, Rehemtulla et al[41] developed an adenoviral vector containing both the therapeutic transgene yeast cytosine deaminase *(yCD)* along with *Fluc*. Following intratumoral injection of the vector into orthotopic 9L gliomas in rats, anatomic and diffusion-weighted MR images were obtained with time to provide for quantitative assessment of overall therapeutic efficacy and spatial heterogeneity of cell kill, respectively. In addition, bioluminescence images assessed the duration and magnitude of gene expression. MR images revealed significant reduction in tumor growth rates associated with *yCD*/5-fluorocytosine (5FC) gene therapy. Significant increases in mean tumor diffusion values were also observed during treatment with 5FC. Moreover, spatial heterogeneity in tumor diffusion changes were also observed, revealing that diffusion MR imaging could detect regional therapeutic effects due to the nonuniform delivery and/or expression of the therapeutic *yCD* transgene within the tumor mass. In addition, bioluminescence imaging in the living mice detected *Fluc* expression, which was found to decrease with time during administration of the prodrug, providing a noninvasive surrogate marker for monitoring gene expression. These results demonstrated the efficacy of the *yCD*/5FC strategy for the treatment of brain tumors and revealed the feasibility of using multitechnique molecular and functional imaging for assessment of gene expression and therapeutic efficacy.

Imaging of Transgenic Models of Spontaneous Disease

The mouse is close to an ideal system to model human diseases because its genome can be easily manipulated and its anatomy and physiology are similar to that of humans. Combinatorial genetic engineering strategies to generate disease-prone genetic strains are now possible to produce new alleles by transgenic technology, in which extra deoxyribonucleic acid (DNA) that encodes the gene of interest is inserted heritably into the mouse genome or by knockout/knockin technology, in which specific portions of the mouse genome are targeted for selective alteration. For example, cyclization recombination (Cre) recombinase is an enzyme used to modify genes and chromosomes. A target region to be deleted in a gene locus can be marked for deletion by signal intensity sequences of locus of X-over P1 (loxP) that are identified by Cre. The expression of

Table 3: Recent examples of applications of molecular neuroimaging in gene therapy

Type of Vector	Location of Imaging Gene, Therapeutic Gene	Imaging Gene Linked to Delivery	Site of Vector	Animal Model	Imaging Method	Application	Ref
Adeno-associated viruses	In delivery vehicle	No	In utero	Mice	Fluc BLI	Evaluation of systemic spread of virus, including to brain, for potential to deliver therapeutic genes to ameliorate genetic diseases with perinatal morbidity and mortality	38
Lentivirus	In delivery vehicle	Yes	Intravenous	Mice	Fluc BLI	Evaluation of gene therapy for Fabry disease using gene for α-galactosidase A, including in brain	39
Nonviral Sleeping Beauty transposon	In target gliomas	No	Brain, intratumoral	Mice	Fluc BLI	Evaluation of antiangiogenesis gene therapy delivered in gliomas; tumor burden assessed with Fluc BLI	40
Adenovirus	In delivery vehicle	Yes	Brain, intratumoral	Rats	Fluc BLI	Evaluation of glioma gene therapy using *yCD*/5-FC	41

Note:—Ref indicates reference; BLI, bioluminescence imaging.

Cre leads to precise removal of the stretch of DNA between the recombinase signal-intensity sequences. With tetracycline-regulated and Cre recombinase–inducible alleles, the timing, duration, and tissue compartment of gene expression or inactivation can be further controlled. These technologies can be combined to yield faithful genetically modified mouse models of specific diseases that overexpress or lack genes of interest in all cells or only in a specific tissue compartment and/or developmental stage of interest.[42]

The strong merits of noninvasive imaging in the assessment of transgenic animals can be readily appreciated from the previous discussion of the overall advantages of molecular imaging in living subjects. More specifically, imaging techniques offer the possibility of adding in vivo phenotyping and monitoring of live animals for diagnostic purposes of disease identification, observing disease progression, and longitudinal effects of drug action, each mouse being its own control.[43] To date, several research groups have used bioluminescence neuroimaging in their assessment of transgenic mice (Table 4).[44-54] For example, Lin et al[46] have imaged transforming growth factor (TGF)-β signaling in living mice in response to brain injury (Fig 3), and Kadurugamuwa et al[51] have developed a method to simultaneously image pneumococcal meningitis and the accompanying neuronal injury (Fig 4). Other examples of transgenic models of spontaneous cancer, in which tumor formation is dependent on defined genetic alterations, provide a powerful test system for evaluating the therapeutic efficacy of pathway-specific antineoplastics. Vooijs et al[54] have generated a conditional mouse model for retinoblastoma-dependent sporadic cancer that permits noninvasive monitoring of pituitary tumor development in living mice by bioluminescence imaging of *Fluc* expression. Bioluminescence imaging of pituitary cancer development with coexpression of the *Fluc* gene enabled longitudinal monitoring of tumor onset, progression, and response to therapy and may be used effectively for testing cancer prevention and treatment strategies on the basis of therapeutics that specifically target the retinoblastoma pathway. More recently, Momota and Holland[55] have developed an imaging approach to measure cell proliferation in transgenic mice harboring gliomas with the *E2F1* promoter (the E2F family are transcription factors) driving expression of *Fluc*. It is known that inactivation of the retinoblastoma protein (RB) pathway is one of the commonest alterations in gliomas as a result of mutations in either the *RB* gene or upstream regulators of phosphor-RB. The *E2F1* promoter is strictly regulated by RB in cell-cycle progression and, in tumor cells, appears to mediate tumor-selective transgene expression. Linkage of the *E2F1* promoter to *Fluc* allows the imaging of genetically induced loss of RB control as a model of human gliomas.

Imaging of Molecular Interactions or Events

Some interesting variations on standard reporter gene assays described previously have also been adapted recently for imaging of molecular interactions in the brains of mice. In particular, imaging interacting protein partners or protein trafficking in mice could pave the way for functional proteomics in whole animals and the assessment of dysfunctional signaling networks in diseased cells and could provide a tool for evaluation of new pharmaceuticals targeted to modulate protein-protein interactions and protein translocation.[56] To this end, Kim et al[57] have developed a genetically encoded bioluminescent indicator for imaging the nuclear trafficking of target proteins in vivo. The principle is based on reconstitution of split fragments of *Renilla* luciferase (Rluc) that are inactive in their split state. The N-terminal fragment of split Rluc is intentionally localized in the nucleus, whereas the C-terminal fragment joined to a particular protein (the androgen receptor in this example) is in the cytosol. Translocation of the receptor (on binding to 5a-dihydrotestosterone [DHT]) into the nucleus results in reconstitution of full-length Rluc and recovering its bioluminescence activity. Thus, imaging and quantifying the occurrence of nucleocytoplasmic trafficking of the androgen receptor was demonstrated after brain implantation of COS-7 cells cotransfected with the genes encoding the receptor and the split Rluc fragments. On delivery of DHT, there was restored bioluminescence signal intensity indirectly indicating the trafficking of the receptor to the nucleus. This was

Table 4: Recent examples of applications of molecular neuroimaging in transgenic animals

Type of Genetically Engineered Model	Promoter Expression	Location of Model	Animal Method	Imaging	Application	Reference
Transgenic	Estrogen receptor	Ubiquitous + brain	Mice	Fluc BLI	Study of estrogen control of growth, differentiation, and function of many systems; study of implications for estrogen-replacement therapy	44
Transgenic	*GFAP*	Brain	Mice	Fluc BLI	Dynamic monitoring of neuronal cell death	45
Transgenic	*Smad* binding element responsive to TGF-β signaling	Brain	Mice	Fluc BLI	Study of *Smad2/3* activation in traumatic brain injury	46
Transgenic	*Smad* binding element responsive to TGF-β signaling	Brain	Mice	Fluc BLI	Study of *Smad2* activation in neuronal degeneration	47
Transgenic	Serum amyloid A protein 1	Ubiquitous + brain	Mice	Fluc BLI	Study of role of *SAA1* induction in chronic inflammation associated with amyloid deposition	48
Gene targeting knockin	CMV	Ubiquitous + brain	Mice	GFP and RFP fluorescence	Study of alternative splicing regulation of *FGFR-2* in the brain.	49
Transgenic	IκBα	Ubiquitous + brain	Mice	Fluc BLI	Study of regulation of *IκBα* expression and *NF-κB* transcriptional activity	50
Transgenic	Mouse *GFAP*	Brain	Mice	Fluc BLI	Study of meningitis and accompanying neuronal injury	51
Transgenic	*c-fos*, CMV	Ubiquitous + brain	Mice	Fluc BLI	Study of immediate-early genes involved in neural pathways linked to specific behaviors	52
Transgenic	Estrogen-responsive elements	Ubiquitous + brain	Mice	Fluc BLI	Study of activation of estrogen receptors and kinetics of gene activation by estrogenic compounds	53
Conditional recombinase knockout	Pro-opiomelanocortin	Pituitary	Mice	Fluc BLI	Study of spontaneous retinoblastoma pathway-dependent pituitary cancer and its response to doxorubicin	54
Transgenic	*E2F1*	Brain	Mice	Fluc BLI	Imaging cell proliferation in gliomas with loss of RB control	55

Note:—BLI indicates bioluminescence imaging; *FGFR-2*, fibroblast growth factor receptor-2; CMV, cytomegalovirus; GFP, green fluorescent protein; RFP, red fluorescent protein; *IκBα*, an inhibitor of nuclear transcription factor *NF-κB*, which regulates the expression of proinflammatory and cytotoxic genes; *c-fos*, an immediate early gene; RB, retinoblastoma protein.

reduced or inhibited on intraperitoneal injection of 2 agents, procymidone and polychlorinated biphenyls, supporting their likely antiandrogenic and neurotoxic effects, respectively. This study could provide a basis for a wide variety of imaging applications for screening drugs or neurotoxic compounds and testing them in preclinical animal models.

Clinical Applications in Molecular Neuroimaging Using Reporter Genes

One expectation of the ongoing developmental research in reporter gene expression imaging exemplified previously might be its straightforward translation from animal work to clinical practice.[58] However, human applications present more theoretic and practical challenges than those in laboratory rodents.[1] This is mostly because of the need for molecular probes to be biocompatible in humans, the presence of many physiologic/morphologic barriers to the delivery of genes and probes, and the need to develop special in vivo amplification strategies for low-level biologic events. Moreover, clinical imaging systems must be capable of obtaining high spatial/temporal resolution images and must be sensitive enough to detect these biologic processes. Because it is necessary to transduce living cells with imaging reporter genes, it follows that many of these practical requirements for successful implementation of reporter gene imaging in patients would mirror many of the logistic requirements and concerns in the field of human gene therapy. In addition, gene therapy is one of the main target areas of reporter gene imaging research because these imaging technologies are anticipated to be of significant help in monitoring transgene expression in a noninvasive manner.

Gene therapy has been one of the great yet unfulfilled promises of recent years. Yet, it has shown slow but steady progress thus far, with many of the obstacles becoming surmountable.[59] Progress in this state of affairs will define mostly to what extent reporter gene expression imaging will translate into clinical practice. Overcoming the hurdles of targeting ex-

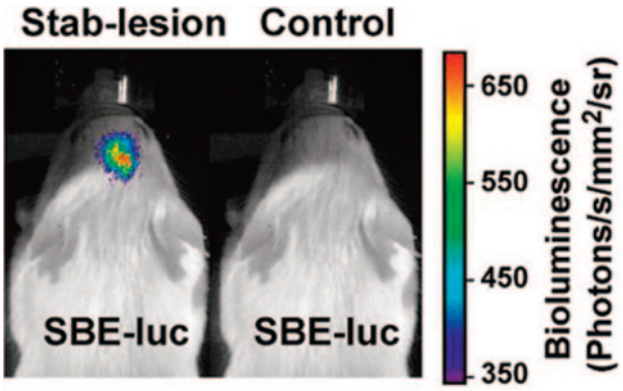

Fig 3. Brain injury results in activation of TGF-β responsive genes and the *Smad*-binding element (*SBE*)-*firefly* luciferase (*luc*) reporter. Two *SBE-luc* mice with similar basal levels of bioluminescence were lesioned with a needle stab to the right hemisphere or were left untreated (control), and bioluminescence was recorded 1 hour later. To highlight the increase in signal intensity in the lesioned mouse, the color scale was adjusted to leave the basal Fluc expression in the control mouse uncolored (<200 photons[p]/s/mm^2/sr). (Copyright 2005, The American Association of Immunologists)

pression of exogenous or endogenous genes to cells or tissue in humans by using imaging reporters for long-term imaging is a theoretic major hurdle at present and will remain so until the current practical challenges of human gene therapy discussed previously are addressed appropriately. Intensive ongoing efforts are also underway to develop alternative simpler strategies for potential future human applications, such as the delivery of circulating exogenous split reporter proteins into cells by using leader peptide sequences. On the other hand and unlike gene therapy, future clinical applications in cell therapy (eg, by using cell-mediated immunotherapy[60] or stem cells[61]) likely stand to benefit considerably and much sooner from reporter gene imaging, as is already clear from studies of cell trafficking in animal experiments. Indeed, Yaghoubi et al[62] have recently demonstrated the first clinical experience in positron-emission tomography (PET) imaging of herpes simplex virus type-1–thymidine kinase (HSV1-*tk*)-expressing autologous cytolytic T lymphocytes directed at recurrent gliomas.

As with gene therapy, one of the challenges facing reporter gene expression imaging is to generate disease- or site-specific imaging strategies. Both the transductional targeting of the vector and the restriction of reporter gene expression solely to the target are potential avenues to follow once translated into clinical practice. Sufficient imaging probe would need to reach the target in vivo to achieve this specificity. Unlike MR imaging and bioluminescence imaging, both PET and SPECT use trace amounts (nonpharmacologic nanogram levels) of molecular probe to obtain images, as is the current practice for clinical scintigraphic imaging. These amounts of molecular probe are known to be safe in humans. More specifically in this regard, Yaghoubi et al[63,64] have studied the 9-[4-fluorine-18 fluoro-3-(hydroxymethyl)butyl]guanine (^{18}F- FHBG) reporter probe used for imaging expression of the HSV1-*tk* reporter gene. They demonstrated good kinetics, biodistribution, stability, dosimetry, and safety of ^{18}F-FHBG in healthy human volunteers, in preparation for future imaging of patients undergoing HSV1-*tk* suicide gene therapy.

Unfortunately, the disadvantages of optical imaging (discussed in the previous article[8]) in terms of translation to clinical practice far outweigh for now its exceedingly advantageous high sensitivity for detecting low-level biologic events. Similarly, the limitations of MR imaging[8] preclude for now its applications in reporter gene imaging of the brain. These issues, therefore, tend to favor the use of PET and SPECT imaging as a viable compromise for clinical implementation of this molecular imaging strategy in neuroimaging, particularly when considering the many merits of these 2 techniques.[8] However, biologic and biophysical factors involved in the biodistribution of reporter probes that are potentially applicable to clinical imaging will also need to be studied carefully as they are scaled up from small animal imaging.

Regrettably, the molecular probes for the HSV1-tk enzyme (both of those based on radiolabeled uracil nucleosides and acycloguanosine derivatives) barely penetrate the intact blood-brain barrier (BBB).[63,65] The BBB is a selective barrier formed by endothelial cells lining cerebral microvessels.[65,66] It acts as a de facto "physical barrier" on account of complex tight junctions between adjacent endothelial cells, forcing most molecular traffic to take a transcellular route across the BBB, rather than moving paracellularly through the junctions, as in most endothelia.[67] This effectively filters most ionized water-soluble molecules >180 Da in molecular weight.[68] Most molecular probes for HSV1-tk are based on the structure of ganciclovir.[69] This has a molecular weight of 255 Da, and it only achieves a concentration in the brain of about 50% of the plasma level. ^{18}F-labeled acycloguanosine derivatives are heavier and usually with extra methyl and fluoro side chains added to the ganciclovir structure.

Not surprisingly, Hospers et al[70] have found in previous biodistribution studies that the uptake of 9-[(3-[^{18}F] fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG) in brain tissue is approximately eightfold lower than the level of FHPG in plasma, reflecting this restricted passage through the intact BBB. On the other hand, the disrupted blood-tumor barrier has been shown in some studies to allow passage of similar probes in experimental rodent intracranial tumors,[14] but this is not a consistent observation (see below findings in the clinical setting). As well, the permeability of the BBB may be altered during cerebral infection (eg, herpes simplex encephalitis) due to the release of chemical mediators such as bradykinin, arachidonic acid, histamine, and free radicals.[71,72] Attempts to modulate the permeability of the BBB pharmacologically have been undertaken to enhance chemotherapeutic drug delivery within the brain. LeMay et al[73] have previously demonstrated that the vasodilatory bradykinin analog RMP-7 increases brain tumor permeability to ganciclovir. It remains to be investigated whether the use of osmotic disruption or RMP-7 may possibly increase delivery of other HSV1-tk substrates across the BBB for molecular neuroimaging purposes.

Unfortunately, once injected systemically, the promiscuous tropism of certain viruses does limit cell-specific gene delivery by these vectors. Viral engineering strategies could ultimately benefit reporter gene expression in the clinical setting, especially to address the tropism of adenoviral vectors.[74] Therefore, local delivery of imaging genes to the brain has been the only means of tissue transduction in the 2 preliminary clinical neuroimaging studies reported so far. Jacobs et al[75] intraoperatively infused liposome vectors carrying the reporter HSV1-*tk* gene directly into tumors during a clinical phase I/II gene therapy trial of 5 patients with

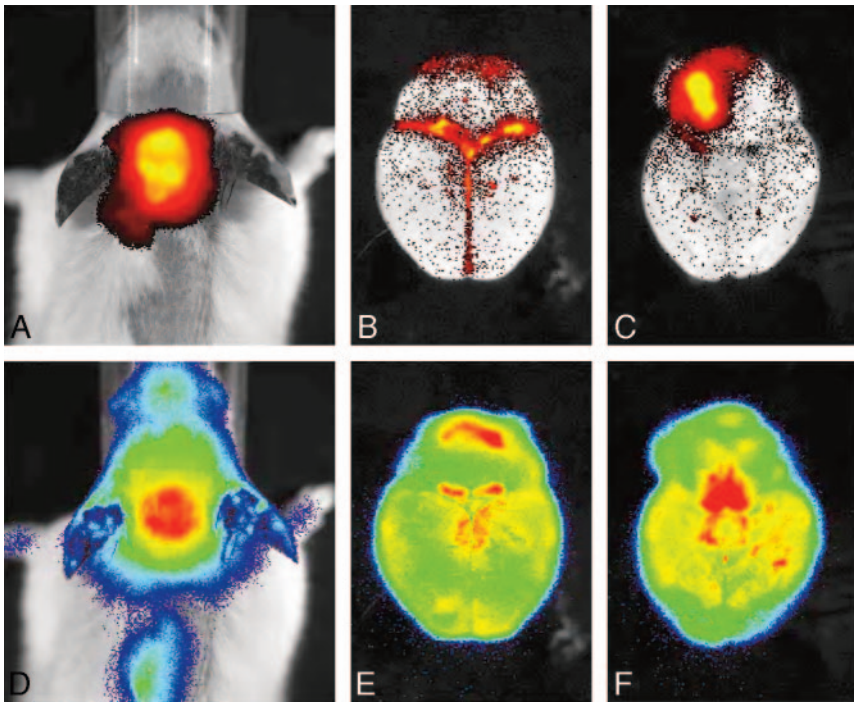

Fig 4. Simultaneous in vivo biphotonic monitoring of pneumococcal meningitis and the accompanying neuronal injury in live transgenic mice. Streptococcus pneumoniae engineered for bioluminescence (*lux*) was used for direct visualization of disease progression. Host response was monitored in transgenic mice containing an inducible *firefly* luciferase (*Fluc*) reporter gene under transcriptional control of the mouse glial fibrillary acidic protein (*GFAP*) promoter. On the basis of the different spectra of light emission and substrate requirements for *Fluc* and *luc*, it is possible to monitor separately the 2 reporters by using a highly sensitive in vivo imaging system. In vivo (*A* and *D*) and ex vivo (*B, C, E,* and *F*) images of brains from transgenic mice with meningitis were obtained at 19 hours postinfection. *A, B,* and *C* show *lux* imaging and *D, E,* and *F* show *Fluc* imaging. Dorsal and ventral views of an ex vivo brain show the bacterial and *GFAP* signals individually. Much of the bacterial signal intensity comes from discrete patches, whereas *GFAP* is induced in the entire brain and there are different intensities of the bioluminescence signal intensity in certain regions of the brain. Note the strong bacterial signal intensity immediately surrounding the inoculation site in the anterior right frontal lobe. (Reprinted by permission from the American Society for Microbiology)

recurrent glioblastoma. Noninvasive primary end point (indirect) molecular imaging of the transduced "tissue dose" of vector-mediated therapeutic gene expression was performed by using the molecular probe 2′-fluoro-2′deoxy-l-beta-D-arabinofuranosyl-5-iodo-uracil (^{124}I-FIAU) and PET. The imaged "tissue dose" of therapeutic gene expression was also correlated with the induced therapeutic effect by secondary end point molecular imaging of the metabolic activity and proliferative activity of the tumors by using [^{18}F]fluorodeoxyglucose (^{18}F-FDG) and ^{11}C-methionine (^{11}C-MET), respectively, and PET. One of the 5 patients demonstrated ^{124}I-FIAU accumulation that was significantly above the prevector baseline and therefore consistent with successful imaging of HSV1-*tk* gene expression in gene therapy in man. Moreover, their findings possibly indicate that PET would be a useful tool to monitor transgene expression in gene therapy clinical trials by using viral vectors.

These early clinical examples also demonstrate the kind of synergy necessary between direct molecular imaging (eg, by using ^{18}F-FDG and ^{11}C-MET) and reporter gene methods in successful establishment of safe and effective gene therapy protocols in clinical practice.[76] In another recent study, Dempsey et al [77] attempted, for the first time, to image expression of HSV 1716 during oncolytic viral therapy in human malignant glioma. ^{123}I-FIAU brain SPECT imaging was undertaken in 8 patients receiving intratumoral injection of virus, but no molecular probe accumulation was detected in these treated gliomas. The authors discussed the many factors that may have contributed to this lack of imaging signal intensity, including impermeability of the BBB, inconsistent disruption of BBB, short half-life of ^{123}I, lower sensitivity of SPECT compared with PET, the use of weak promoters, the need for more sensitive molecular probes, possible insufficient viral replication, and potential for improved administration of virus (eg, by using convection enhanced delivery). Nonetheless, this study was useful in highlighting the possible limitations of this technique and the many potential areas that need to be investigated in future research.

Future Outlook

In this article, we discussed the principles and recent technologic advances in molecular imaging of reporter gene expression in the brain. This approach is emerging as a valuable tool for monitoring the expression of genes in laboratory animals and humans. Further development of newer (eg, *Gaussia* luciferase[78]) and more sensitive and selective reporters (eg, red-shifted Rluc, with greater stability and higher light emission than native Rluc[79]), combined with improvements in detection technology, will consolidate the position of reporter gene imaging as a versatile method for understanding of intracellular biologic processes and the molecular basis of neurologic disorders.

Many developments in reporter gene expression imaging are anticipated during the next decade. Significant conceptual and technologic advances will most likely be seen across the 5 main general requirements for molecular imaging discussed previously and in greater detail elsewhere,[1]—that is, knowledge of molecular targets, availability of molecular probes, overcoming delivery barriers, developing amplification strategies, and availability of appropriate instrumentation. In particular, new strategies to circumvent the normal BBB or that target a blood-tumor barrier by the use of novel carrier vehicles (eg, in rabbits, various nutrient transporters continue to be tested across the blood-retinal barrier [used as a model of the BBB] to enhance drug bioavailability across membranes with poor permeability[80]) and local vasodilation or osmotic opening all merit attention, as well as the design of newer reporter gene/probe systems tailored to molecular neuroimaging. As an example of the latter, Majumdar et al[81] have tested modified dipeptide monoester ganciclovir prodrugs for their greater solubility and permeability. On the other hand, Ma-

grassi et al[82] exploited the fact that HSV1-tk is not enantioselective and can therefore efficiently phosphorylate both D and L enantiomers of β-thymidine. Using autoradiography, they showed that tritiated l-β-thymidine is selectively retained to a significant extent in experimental intracranial gliomas. It has the advantage of generating less-toxic metabolites than with use of conventional HSV1-tk probes; and with appropriate radioisotopic labeling (eg, with ^{11}C) of l-thymidine, it might be possible to adapt it for future use in PET studies of the brain.

Another study that exemplifies a search for new reporter systems with capabilities of imaging processes behind an intact BBB is that of Doubrovin et al,[83] who investigated *Escherichia coli* xanthine phosphoribosyltransferase for nuclear imaging with radiolabeled xanthine. Again, by using autoradiography, they found that ^{14}C xanthine was capable of specific accumulation in transfected infiltrative brain tumors. These and future similar innovations bode well for more widespread experimental and potential clinical applications of reporter gene imaging in the brain.

The merger of molecular biology and medical imaging is facilitating rapid growth of this new field by providing methods to monitor an ever-increasing number of cellular/molecular events adapted from conventional molecular assays, including reporter gene assays. Further developments will provide us with the ability to perform simultaneous imaging of multiple molecular events in 1 population of cells in living subjects. This may be attainable by combining 2 or more of the previously described strategies for gene marking and imaging the trafficking of cells with those entailing linked expression of an imaging gene to an endogenous promoter or to an exogenous therapeutic gene. As such, in these applications, it is foreseeable that 1 reporter may reveal the spatial distribution of cells and whether they have reached a specific target, and another reporter may indicate whether a certain gene becomes upregulated at this site or if a more complex interaction occurs. These endeavors will be aided by the availability of multiple fusion reporter constructs (eg, those that combine PET/bioluminescence/fluorescence imaging capabilities in 1 gene),[84] the use of which should accelerate the validation of reporter gene approaches developed in cell culture for translation into preclinical models and subsequent clinical imaging of neurologic disorders. With continued rapid advancements in this field, the experimental and clinical neurosciences stand to gain considerably from noninvasive molecular imaging of the expression of multiple fused reporter genes by using multiple imaging techniques.[85] These approaches are likely to play an increasingly important role in defining molecular events in the field of cancer biology, cell biology, and gene therapy within the central nervous system.

References

1. Massoud TF, Gambhir SS. **Molecular imaging in living subjects: seeing fundamental biological processes in a new light**. *Genes Dev* 2003;17:545–80
2. Weissleder R, Mahmood U. **Molecular imaging**. *Radiology* 2001;219:316–33
3. Jaffer FA, Weissleder R. **Molecular imaging in the clinical arena**. *JAMA* 2005;16:855–62
4. Gross S, Piwnica-Worms D. **Molecular imaging strategies for drug discovery and development.** *Curr Opin Chem Biol* 2006;10:334–42
5. Blasberg RG, Tjuvajev JG. **Molecular-genetic imaging: current and future perspectives.** *J Clin Invest* 2003;111:1620–29
6. Gambhir SS, Barrio JR, Herschman HR, et al. **Assays for noninvasive imaging of reporter gene expression.** *Nucl Med Biol* 1999;26:481–90
7. Blasberg R. **Imaging gene expression and endogenous molecular processes: molecular imaging**. *J Cereb Blood Flow Metab* 2002;22:1157–64
8. Massoud TF, Singh A, Gambhir SS. **Noninvasive molecular neuroimaging using reporter genes. Part I, Principles revisited**. *AJNR Am J Neuroradiol* 2008; 29:229–34
9. Brenner M. **Gene marking.** *Hum Gene Ther* 1996;7:1927–36
10. Rehemtulla A, Stegman LD, Cardozo SJ, et al. **Rapid and quantitative assessment of cancer treatment response using in vivo bioluminescence imaging.** *Neoplasia* 2000;2:491–95
11. Deroose CM, Reumers V, Gijsbers R, et al. **Noninvasive monitoring of long-term lentiviral vector-mediated gene expression in rodent brain with bioluminescence imaging.** *Mol Ther* 2006;14:423–31. Epub 2006 Jul 3
12. Pike L, Petravicz J, Wang S. **Bioluminescence imaging after HSV amplicon vector delivery into brain**. *J Gene Med* 2006;8:804–13
13. Burgos JS, Rosol M, Moats RA, et al. **Time course of bioluminescent signal in orthotopic and heterotopic brain tumors in nude mice.** *Biotechniques* 2003;34:1184–88
14. Cho SY, Ravasi L, Szajek LP, et al. **Evaluation of (76)Br-FBAU as a PET reporter probe for HSV1-tk gene expression imaging using mouse models of human glioma.** *J Nucl Med* 2005;46:1923–30
15. Szentirmai O, Baker CH, Lin N, et al. **Noninvasive bioluminescence imaging of luciferase expressing intracranial U87 xenografts: correlation with magnetic resonance imaging determined tumor volume and longitudinal use in assessing tumor growth and antiangiogenic treatment effect.** *Neurosurgery* 2006;58:365–72
16. Kemper EM, Leenders W, Kusters B, et al. **Development of luciferase tagged brain tumour models in mice for chemotherapy intervention studies.** *Eur J Cancer* 2006;42:3294–303
17. Chaudhari AJ, Darvas F, Bading JR, et al. **Hyperspectral and multispectral bioluminescence optical tomography for small animal imaging.** *Phys Med Biol* 2005;50:5421–41
18. Yang M, Baranov E, Moossa AR, et al. **Visualizing gene expression by whole-body fluorescence imaging.** *Proc Natl Acad Sci U S A* 2000;97:12278–82
19. Buursma AR, de Vries EF, Garssen J, et al. [**18F**]**FHPG positron emission tomography for detection of herpes simplex virus (HSV) in experimental HSV encephalitis.** *J Virol* 2005;79:7721–27
20. Rubin JB, Kung AL, Klein RS, et al. **A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors.** *Proc Natl Acad Sci U S A* 2003;100:13513–18
21. Prins RM, Bruhn KW, Craft N, et al. **Central nervous system tumor immunity generated by a recombinant listeria monocytogenes vaccine targeting tyrosinase related protein-2 and real-time imaging of intracranial tumor burden.** *Neurosurgery* 2006;58:169–78
22. Moriyama EH, Bisland SK, Lilge L, et al. **Bioluminescence imaging of the response of rat gliosarcoma to ALA-PpIX-mediated photodynamic therapy.** *Photochem Photobiol* 2004;80:242–49
23. Hsu AR, Hou LC, Veeravagu A, et al. **In vivo near-infrared fluorescence imaging of integrin alphavbeta3 in an orthotopic glioblastoma model.** *Mol Imaging Biol* 2006;8:315–23
24. Becker W, Meller J **The role of nuclear medicine in infection and inflammation**. *Lancet Infect Dis* 2001;1:326–33
25. Chemaly ER, Yoneyama R, Frangioni JV, et al. **Tracking stem cells in the cardiovascular system**. *Trends Cardiovasc Med* 2005;15:297–302
26. Luker KE, Hutchens M, Schultz T, et al. **Bioluminescence imaging of vaccinia virus: effects of interferon on viral replication and spread.** *Virology* 2005;341: 284–300. Epub 2005 Aug 10
27. Cook SH, Griffin DE. **Luciferase imaging of a neurotropic viral infection in intact animals.** *J Virol* 2003;77:5333–38
28. Luker GD, Bardill JP, Prior JL, et al. **Noninvasive bioluminescence imaging of herpes simplex virus type 1 infection and therapy in living mice.** *J Virol* 2002;76:12149–61
29. Franke-Fayard B, Janse CJ, Cunha-Rodrigues M, et al. **Murine malaria parasite sequestration: CD36 is the major receptor, but cerebral pathology is unlinked to sequestration.** *Proc Natl Acad Sci U S A* 2005;102:11468–73
30. Jenkins DE, Hornig YS, Oei Y, et al. **Bioluminescent human breast cancer cell lines that permit rapid and sensitive in vivo detection of mammary tumors and multiple metastases in immune deficient mice.** *Breast Cancer Res* 2005;7:R444–54
31. Tang Y, Shah K, Messerli SM, et al. **In vivo tracking of neural progenitor cell migration to glioblastomas.** *Hum Gene Ther* 2003;14:1247–54
32. Shah K, Bureau E, Kim DE, et al. **Glioma therapy and real-time imaging of neural precursor cell migration and tumor regression.** *Ann Neurol* 2005;57:34–41
33. Kim DE, Schellingerhout D, Ishii K, et al. **Imaging of stem cell recruitment to ischemic infarcts in a murine model**. *Stroke* 2004;35:952–57
34. Kim DE, Tsuji K, Kim YR, et al. **Neural stem cell transplant survival in brains of mice: assessing the effect of immunity and ischemia by using real-time bioluminescent imaging.** *Radiology* 2006;241:822–30
35. Okada S, Ishii K, Yamane J, et al. **In vivo imaging of engrafted neural stem cells:**

its application in evaluating the optimal timing of transplantation for spinal cord injury. *FASEB J* 2005;19:1839–41. Epub 2005 Sep 1
36. Min JJ, Gambhir SS. **Gene therapy progress and prospects: noninvasive imaging of gene therapy in living subjects.** *Gene Ther* 2004;11:115–25
37. Ray P, Bauer E, Iyer M, et al. **Monitoring gene therapy with reporter gene imaging**. *Semin Nucl Med* 2001;31:312–20
38. Lipshutz GS, Gruber CA, Cao Y. **In utero delivery of adeno-associated viral vectors: intraperitoneal gene transfer produces long-term expression.** *Mol Ther* 2001;3:284–92
39. Yosmitsu M, Sato T, Tao K, et al. **Bioluminescent imaging of a marking transgene and correction of Fabry mice by neonatal injection of recombinant lentiviral vectors.** *Proc Natl Acad Sci U S A* 2004;101:16909–14
40. Ohlfest JR, Demorest ZL, Motooka Y, et al. **Combinatorial antiangiogenic gene therapy by nonviral gene transfer using the sleeping beauty transposon causes tumor regression and improves survival in mice bearing intracranial human glioblastoma.** *Mol Ther* 2005;12:778–88. Epub 2005 Sep 16
41. Rehemtulla A, Hall DE, Stegman LD, et al. **Molecular imaging of gene expression and efficacy following adenoviral-mediated brain tumor gene therapy.** *Mol Imaging* 2002;1:43–55
42. Sharpless NE, DePinho RA. **The mighty mouse: genetically engineered mouse models in cancer drug development.** *Nat Rev Drug Discov* 2006;5:741–54
43. Nieman BJ, Bock NA, Bishop J, et al. **Magnetic resonance imaging for detection and analysis of mouse phenotypes.** *NMR Biomed* 2005;18:447–68
44. Ciana P, Raviscioni M, Mussi P, et al. **In vivo imaging of transcriptionally active estrogen receptors.** *Nat Med* 2003;9:82–86
45. Zhu L, Ramboz S, Hewitt D, et al. **Non-invasive imaging of GFAP expression after neuronal damage in mice.** *Neurosci Lett* 2004;367:210–12
46. Lin AH, Luo J, Mondshein LH, et al. **Global analysis of Smad2/3-dependent TGF-beta signaling in living mice reveals prominent tissue-specific responses to injury.** *J Immunol* 2005;175:547–54
47. Luo J, Lin AH, Masliah E, et al. **Bioluminescence imaging of Smad signaling in living mice shows correlation with excitotoxic neurodegeneration.** *Proc Natl Acad Sci U S A* 2006;103:18326–31
48. Zhang N, Ahsan MH, Purchio AF, et al. **Serum amyloid A-luciferase transgenic mice: response to sepsis, acute arthritis, and contact hypersensitivity and the effects of proteasome inhibition.** *J Immunol* 2005;174:8125–34
49. Bonano VI, Oltean S, Brazas RM, et al. **Imaging the alternative silencing of FGFR2 exon IIIb in vivo.** *RNA* 2006;12:2073–79. Epub 2006 Oct 26
50. Zhang N, Ahsan MH, Zhu L, et al. **Regulation of IkappaBalpha expression involves both NF-kappaB and the MAP kinase signaling pathways.** *J Inflamm (Lond)* 2005;2:10
51. Kadurugamuwa JL, Modi K, Coquoz O, et al. **Reduction of astrogliosis by early treatment of pneumococcal meningitis measured by simultaneous imaging, in vivo, of the pathogen and host response.** *Infect Immun* 2005;73:7836–43
52. Collaco AM, Geusz ME. **Monitoring immediate-early gene expression through firefly luciferase imaging of HRS/J hairless mice.** *BMC Physiol* 2003; 3:8. Epub 2003 Aug 19
53. Lemmen JG, Arends RJ, van Boxtel AL, et al. **Tissue- and time-dependent estrogen receptor activation in estrogen reporter mice.** *J Mol Endocrinol* 2004;32:689–701
54. Vooijs M, Jonkers J, Lyons S, et al. **Noninvasive imaging of spontaneous retinoblastoma pathway-dependent tumors in mice.** *Cancer Res* 2002;62:1862–67
55. Momota H, Holland EC. **Bioluminescence technology for imaging cell proliferation.** *Curr Opin Biotechnol* 2005;16:681–86
56. Massoud TF, Paulmurugan R, De A, et al. **Reporter gene imaging of protein-protein interactions in living subjects.** *Curr Opin Biotechnol* 2007;18:31–37
57. Kim SB, Ozawa T, Watanabe S, et al. **High-throughput sensing and noninvasive imaging of protein nuclear transport by using reconstitution of split Renilla luciferase.** *Proc Natl Acad Sci U S A* 2004;101:11542–47. Epub 2004 Aug 2
58. Pomper MG. **Translational molecular imaging for cancer**. *Cancer Imaging* 2005;5:S16–S26
59. Somia N, Verma IM. **Gene therapy: trials and tribulations**. *Nat Rev Genet* 2000;1:91–99
60. Lucignani G, Ottobrini L, Martelli C, et al. **Molecular imaging of cell-mediated cancer immunotherapy.** *Trends Biotechnol* 2006;24:410–18
61. Swijnenburg RJ, van der Bogt KE, Sheikh AY, et al. **Clinical hurdles for the transplantation of cardiomyocytes derived from human embryonic stem cells: role of molecular imaging.** *Curr Opin Biotecnol* 2007;18:38–45
62. Yaghoubi S, Jensen MC, Wang Y, et al. **Imaging of HSV1-tk expressing cytolytic T cell clones in glioma patients with [^{18}F]FHBG positron emission tomography.** *Mol Ther* 2007;15(suppl 1):S229
63. Yaghoubi S, Barrio JR, Dahlbom M, et al. **Human pharmacokinetic and dosimetry studies of [(18)F]FHBG: a reporter probe for imaging herpes simplex virus type-1 thymidine kinase reporter gene expression.** *J Nucl Med* 2001;42:1225–34
64. Yaghoubi SS, Couto MA, Chen CC, et al. **Preclinical safety evaluation of 18F-FHBG: a PET reporter probe for imaging herpes simplex virus type 1 thymidine kinase (HSV1-tk) or mutant HSV1-sr39tk's expression.** *J Nucl Med* 2006;47:706–15
65. Abbott NJ, Chugani DC, Zaharchuk G, et al. **Delivery of imaging agents into brain.** *Adv Drug Deliv Rev* 1999;37:253–77
66. Abbott NJ, Ronnback L, Hansson E, et al. **Astrocyte-endothelial interactions at the blood-brain barrier.** *Nat Rev Neurosci* 2006;7:41–53
67. Wolburg H, Lippoldt A. **Tight junctions of the blood-brain barrier: development, composition and regulation.** *Vascul Pharmacol* 2002;38:323–37
68. Kroll RA, Neuwelt EA. **Outwitting the blood-brain barrier for therapeutic purposes: osmotic opening and other means.** *Neurosurgery* 1998;42:1083–99
69. Iyer M, Barrio JR, Namavari M, et al. **8-[18F]Fluoropenciclovir: an improved reporter probe for imaging HSV1-tk reporter gene expression in vivo using PET.** *J Nucl Med* 2001;42:96–105
70. Hospers GAP, Calogero A, van Waarde A, et al. **Monitoring of herpes simplex virus thymidine kinase enzyme activity using positron emission tomography.** *Cancer Res* 2000;60:1488–91
71. Bidanset DJ, Placidi L, Rybak R, et al. **Intravenous infusion of cereport increases uptake and efficacy of acyclovir in herpes simplex virus-infected rat brains.** *Antimicrob Agents Chemother* 2001;45:2316–23
72. Wahl M, Unterberg A, Baethmann A, et al. **Mediators of blood-brain barrier dysfunction and formation of vasogenic brain edema.** *J Cereb Blood Flow Metab* 1988;8:621–34
73. LeMay DR, Kittaka M, Gordon EM, et al. **Intravenous RMP-7 increases delivery of ganciclovir into rat brain tumors and enhances the effects of herpes simplex virus thymidine kinase gene therapy.** *Hum Gene Ther* 1998;9:989–95
74. Urbain JL. **Reporter genes and imagine**. *J Nucl Med* 2001;42:106–09
75. Jacobs A, Voges J, Reszka R, et al. **Positron-emission tomography of vector-mediated gene expression in gene therapy for gliomas.** *Lancet* 2001;358:727–29
76. Jacobs AH, Winkeler A, Dittmar C, et al. **Prospects of molecular imaging in neurology.** *J Cell Biochem Suppl* 2002;39:98–109
77. Dempsey MF, Wyper D, Owens J, et al. **Assessment of 123I-FIAU imaging of herpes simplex viral gene expression in the treatment of glioma.** *Nucl Med Commun* 2006;27:611–17
78. Venisnik KM, Olafsen T, Gambhir SS, et al. **Fusion of Gaussia luciferase to an engineered anti-carcinoembryonic antigen (CEA) antibody for in vivo optical imaging.** *Mol Imaging Biol* 2007;9:267–77
79. Loening AM, Wu AM, Gambhir SS. **Red-shifted *Renilla reniformis* luciferase variants for imaging in living subjects.** *Nat Methods* 2007;4:641–43. Epub 2007 Jul 8
80. Duvvuri S, Majumdar S, Mitra AK. **Drug delivery to the retina: challenges and opportunities.** *Expert Opin Biol Ther* 2003;3:45–56
81. Majumdar S, Nashed YE, Patel K, et al. **Dipeptide monoester ganciclovir prodrugs for treating HSV-1-induced corneal epithelial and stromal keratitis: in vitro and in vivo evaluations.** *J Ocul Pharmacol Ther* 2005;21:463–74
82. Magrassi L, Finocchiaro G, Milanesi G, et al. **Lack of enantioselectivity of herpes virus thymidine kinase allows safer imaging of gene delivery.** *Gene Ther* 2003;10:2052–58
83. Doubrovin M, Ponomarev V, Serganova I, et al. **Development of a new reporter gene system–dsRed/xanthine phosphoribosyltransferase-xanthine for molecular imaging of processes behind the intact blood-brain barrier.** *Mol Imaging* 2003;2:93–112
84. Ray P, De A, Min JJ, et al. **Imaging tri-fusion multimodality reporter gene expression in living subjects.** *Cancer Res* 2004;64:1323–30
85. Massoud TF, Gambhir SS. **Integrating noninvasive molecular imaging into molecular medicine: an evolving paradigm.** *Trends Mol Med* 2007;13:183–91