EDITORIAL

Triumph over Mischance: A Role for Nuclear Medicine in Gene Therapy

Yield not thy neck to fortune's yoke, but let thy dauntless mind still ride in triumph overall mischance.
King Henry the Sixth, W.H. Shakespeare

The human genome has about 100,000 individual genes. These genes produce the macromolecules that form, in large measure, our human potential for sickness or for health. In the last decade our knowledge of genetics has progressed rapidly and we have learned that not only do we obtain our genetic inheritance at the moment of conception but genetic alterations during life are the basis for many important human diseases. In particular, common human cancers appear to develop from a series of somatic cell mutations that are most probably due to environmental insults occurring at susceptible points in the genome. This "multihit" hypothesis has been most thoroughly developed for colorectal cancer by Vogelstein and his colleagues (*1*), and damaged genes with an important role in human cancer, such as the mutated p53 gene, play a major role in carcinogenesis.

Gene therapy is the manipulation of the expression of genes in human cells for the purpose of treating genetically-based human disease. It has actually become possible to treat some diseases by repairing the damaged gene or, in other cases, altering genes to destroy damaged cells. A recent review describes the concepts and terminology of this rapidly emerging discipline (*2*).

Radiolabeled peptides that bind to specific cell-associated receptors have shown great promise in selectively targeting human tumors, diagnosing tumors, characterizing the tumor cell biology in terms of receptor expression, and targeting radiotherapy. The pioneering work of Reubi, Krenning, Lamberts and others on the use of somatostatin analogs, labeled with ^{111}In (*3*), has set the stage for these developments. Indium-111-octreotide now is used widely for diagnosing APUDomas, as well as other tumors of neuroendocrine origin. Other peptides, such as radiolabeled VIP, offer similar promise for diagnosing gastrointestinal malignancies (*4*).

In an accompanying article, Rogers et al. (*5*) describe a gene transfer-mediated approach to improving targeting of radiolabeled bombesin peptide to tumor, with the ultimate purpose that the radiolabeled peptide would be a carrier molecule for antitumor therapy using target radionuclides, such as ^{125}I. Although not emphasized by the authors, this technique does offer an approach to detecting the presence of an activated gene in vivo through localizing a specific ligand to the gene product, the bombesin receptor. This paper offers an intriguing example of how advances in tumor genetics can be combined with advances in nuclear medicine to provide tumor targeting of radiolabeled molecules based on a specific membrane receptor produced by induction of genes that are not normally expressed in the target cell.

The use of radiotracers to target genetically altered cells in vivo is still in its infancy, and no human studies have as yet been reported. The successful animal studies are listed in Table 1 (*5–16*). The studies are of two general types: (a) targeting due to metabolic trapping of a specific substrate (marker substrate), based on a specific genetic alteration (marker gene) and (b) targeting to a cell membrane receptor, which is induced artificially by genetic engineering. These preliminary data suggest a vast potential for nuclear medicine applications in monitoring gene expression in vivo in gene therapy studies.

GENE THERAPY

A limitation of many biological-based therapies has been our inability to achieve controlled and effective delivery of biologically active molecules to tumor cells or their surrounding matrix. This condition is particularly limiting for biologically active compounds that have very short half-lives and exhibit site-specific therapeutic and toxic effects. Gene-based therapy can provide control over the level, timing and duration of action of these biologically active products by including specific promoter/activator elements in the genetic material transferred resulting in more effective therapeutic interventions. Methods are actively being developed for controlled gene delivery to various somatic tissues and tumors using novel formulations of DNA, and for controlling gene expression using cell specific, replication-activated and drug-controlled expression systems (*17*). Targeting gene therapy to particular tissue (e.g., tumor) or specific organs is an increasingly active area of research with over 400 related articles published in 1994, over 800 articles in 1995 and over 1200 articles in 1996.

Current gene-based therapies for cancer involve several different biological approaches to treatment and new approaches continue to be developed. There are over 140 human gene-therapy clinical trials currently being investigated in the U.S. (*18*), and additional studies are being performed in Europe and Asia. Included are 64 "immunotherapy/cytokine" protocols, most of which involve the transfer of cytokine genes into tumor cells. There are 15 "tumor suppressor (antioncogene)/antisense" protocols. There are seven "drug resistance" protocols, all of which involve transfer of multidrug resistance cDNA. There are 32 "gene marker" protocols that are primarily designed to assess the efficacy and safety of gene transfer and gene therapy in patients. Most of these protocols involve transfer of the neomycin-resistance gene into lymphocytes or stem cells ex-vivo and their readministration to patients. Questions related to the distribution and persistence of the genetically altered cells, identified by their resistance to neomycin toxicity, are being addressed.

There are also 26 "drug sensitivity" protocols; 25 involve retroviral transfer of the HSV1-tk gene into tumor cells followed by systemic treatment with ganciclovir. Their use in cancer therapy is to create significant differences between normal and malignant cells by selective transduction of the "susceptibility" gene into malignant, but not normal cells. This approach is based on the fact that certain genes can be used to sensitize cells to drugs that are normally inactive or nontoxic (e.g., prodrug), and these genes have been described as "susceptibility" or "suicide" genes. Most susceptibility genes encode viral or bacterial enzymes that convert inactive forms of a drug,

Received Mar. 11, 1997; accepted Mar. 16, 1997.
For correspondence or reprints contact: Steven M. Larson, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

TABLE 1
Marker Gene/Marker Substance Studies

Gene	Radiotracer (route)	Target tissue	Host (site)	Transfection mode	Vector type	Reference
HSV-tk	FIAU* $^{131}I/^{124}I$ (IV)	RG2/W256 tumor	Rodent (flank)	In vivo/in vitro	gpSTK-A2 retroviral	Tjuvajev et al. (*6–10*)
HSV-tk	FIVAU, FIVRU ^{131}I (IV)†	Balb STK	Rodent (flank)	In vitro	STK retrovirus	Morin et al. (*11–13*)
HSV-tk	^{18}F-acyclovir‡	Liver	Rodent	In vivo	Adenovirus	Srinivasan et al. (*14*)
HSV-tk	$^{18}FFHPG$¶¶	9L glioma	Rodent (brain)	In vivo	—	Goldman et al. (*15*)
GRPr%	[^{125}I]-miP-bombesin; ^{125}I-Tyr^4-bombesin	SKOV3.ip1 tumor	Peritoneum	In vivo	Adenovirus (AdCMVGRPr)	Rogers et al. (*5*)

*Percent gastrin-releasing peptide receptor.
†Fluoro-1 b D arabinofuranosyl-5-[I-131]-iodo-uracil.
‡2'-fluoro-5-(iodovinyl)-arabinofuranosyl- and -ribofuranosyl-iodo-uracil.
§Fluorine-18-fluoroacyclovir.
¶¶9-1 {(1-[^{18}F]fluoro-3-hydroxy-2-propoxy)methyl} guanine.

prodrug, into toxic compounds or antimetabolites capable of inhibiting nucleic acid synthesis. HSV thymidine kinase selectively converts the normally nontoxic prodrug, ganciclovir, into toxic compounds that results in cell death. These so-called "drug sensitivity" or "susceptibility" gene therapy protocols depend on the expression of HSV-tk in the target (tumor) tissue. These protocols are summarized in Table 2.

TYPICAL PROTOCOL DESIGN

The in vivo gene therapy can be illustrated by an example of a prodrug activating gene therapy protocol. A typical pro-drug activating gene therapy protocol (i.e., HSV1-tk or E. Coli cytosine deaminase) includes CT- or MRI-guided multiple intratumoral injections of a suspension containing the retroviral vector producer cells or adenoviral vectors. The adenoviral vectors infect and express the transgene in both proliferating and nonproliferating cells. A peak of gene expression after adenoviral-mediated gene transfer occurs at 1–3 days postinoculation. The retroviral vectors integrate only into the genome of proliferating cells, but the expression of transgene in not proliferation dependent. Implantation of the retroviral vector producer cells provides a local source for sustained supply of retroviral vector and a larger magnitude of transduction of proliferating tumor cells. Therefore, in case of HSV1-tk gene the administration of ganciclovir is initiated 1–3 days after adenoviral-mediated gene transfer or 2 wk after implantation of the retroviral vector producer cells. A usual duration of ganciclovir treatment is 2 wk. After the completion of ganciclovir administration, patients are followed up for several months to assess the effect of prodrug activating gene therapy by MRI. Thus, the efficacy of gene transfer and expression as well as the results of prodrug activation therapy can be assessed only after several weeks or even months by measuring changes in tumor size.

Potential Clinical Impact of Noninvasive Imaging of Transduced Genes

Several issues that are important for clinical optimization of gene therapy remain unresolved in many current clinical protocols:

1. Has gene transduction or transfection been successful?
2. Is the distribution of the transduced or transfixed gene localized to the target organ or to target tissue, and is the distribution in the target optimal?
3. Is the level of gene expression in the target organ or tissue sufficient to result in a therapeutic effect?
4. In the case of combined prodrug-gene therapy protocols, when is gene expression maximum (optimal) and when is the optimal time to initiate treatment with the prodrug? and
5. How long does gene expression persist in the target and other tissues? As an aid to answering these questions, a noninvasive, clinically-applicable method for imaging the expression of successful gene transduction in target tissue or specific organs of the body would be of considerable value. It would facilitate the monitoring and evaluation of gene therapy in human subjects by defining the location, magnitude and persistence of gene expression over time.

NUCLEAR MEDICINE APPROACHES

Marker Substance/Marker Gene Approach

Clinical monitoring of gene expression requires the appropriate combination of "marker gene" and "marker substrate." We consider the following characteristics to be ideal, if not essential (*7*):

1. A "marker gene" is usually a "foreign gene" that is not present (or not normally expressed) in the host

TABLE 2
Gene Therapy Trials Worldwide

Trials	Number
Cytokine/Immunotherapy	64
· Carcinoembryonic antigen	3
· Prostate-specific antigen	1
· HLA-B7	8
· HLA-B7 and β-2 microglobulin	4
· IL-2	25
· IL-2 and β-galactosidase (*E. Coli*)	1
· IL-2 sense and TGF-β2 antisense	1
· IL-4	4
· IL-7	2
· IL-12	1
· GM-CSF	4
· INF-g	2
· INF-g and IL-2	1
· IL-7 and IL-12 and GM-CSF	2
· TNF (cDNA)	1
· Ig (cDNA)	1
· T-cell receptor antibody	1
· MART-1	1
· IGF-1 antisense	1
Tumor suppressor	15
· p53 antisense	3
· p53 sense	4
· Retinoblastoma	1
· *c-fos* and/orc-*myc*	2
· *c-myb*	1
· *bcr/abl* antisense	1
· E1A	1
· BRCA-1	1
· Anti-erB-2 single-chain antibody	1
Drug resistance	7
· Multiple drug resistance (MDR)	6
· MDR and neomycine resistance	1
Marker gene	32
· *E. Coli* β-galactosidase	2
· Neomycine resistance	30
Drug sensitivity	26
· HSV1-tk	24
· *E. Coli* cytosine deaminase	2

tissue, and the "gene product" is an enzyme that can be expressed in transduced or transfected host cells. This enzyme must be nontoxic to host cells and catalyze a reaction with the "marker substrate" where the reaction product accumulates within transduced or transfected cells.

2. A "marker substrate" is chosen to match the "marker gene." It is a compound that is not metabolized (or only slowly metabolized) by the host, and does not accumulate in nontransduced or nontransfected host tissue. It can be radiolabeled with appropriate isotopes for clinical imaging using gamma camera, SPECT or PET techniques, or is a paramagnetic compound appropriate for clinical MR imaging.
3. The "marker substrate" must cross cell membranes readily, be rapidly metabolized by the "marker gene" product and be effectively trapped within transduced cells throughout the period of imaging, and it must accumulate to levels that are measurable by existing clinical imaging techniques.
4. The accumulation of the "marker substrate" in transduced or transfected cells must reflect the activity of the "gene product" and, thereby, the expression of the "marker gene" in transduced or transfected tissue. Animal studies show the feasibility of imaging gene expression using nuclear medicine techniques (Table 1).

The studies of Tjuvejev et al. (*6*) are illustrative of the optimization of a noninvasive imaging system based on the concept of marker substrate/marker gene. This approach has used a marker substance (radiolabeled FAIU) and a marker gene (HSV1-tk) to noninvasively monitor the transfection of human xenograft in vivo. Noninvasive imaging of gene expression has the potential to greatly facilitate gene therapy studies by monitoring in vivo and noninvasively, the expression of the gene, as a function of time post-transfection and also the persistence of the genes in vivo. In addition, since the uptake is correlated with gene expression and subsequent sensitivity to drugs, this will also allow establishing dose response relationships at the level of the cell. In this successful, noninvasive imaging of herpes simplex virus Type 1, thymidine kinase (HSV1-tk) expression was demonstrated by the gamma camera and by SPECT using ^{131}I 2′-fluoro-2′-deoxy-1-b-D-arabinofuranosyl-5-iodo-uracil (FIAU). Studies were performed in rats bearing wild type and STK retrovirus transfected RG2 glioma and Walker 256 mammary carcinomas, both in vitro and in vivo by injection of the retroviral vector producing gp-STK-A2 cells. Imaging was performed 2–3 wk after tumor transduction to allow time for production and spread of the retroviruses through the tumor and for sufficient growth and increase in size of the tumor to facilitate imaging. One to two millicuries of [^{131}I]-FIAU was used in this instance. Uptake correlated with in vitro ganciclovir sensitivity.

In comparison to conventional gamma camera imaging, PET offers higher resolution and quantitative uptake measurements in vivo. Recently, HSV1-tk transduction in rat tumors was demonstrated with [^{124}I]-FIAU and PET (*9*), and in the future PET will likely be the method of choice for such gene imaging studies (*14*).

In most instances the marker substance is a nucleoside that is metabolically trapped in the cell in the presence of the HSV1-tk marker gene (*6–16*). It is likely that improved nucleoside targeting agents will be developed. It is noted that there are derivatives of the FAIU compound that may have even better imaging characteristics than FIAU, and will be more resistant to metabolic breakdown. Promising marker substances include FIVAU and FIVRU, which are the 2′-fluoro-5-(iodovinyl)-arabinofuranosyl- and the ribofuranosyl analogs (*11–13*). Other investigators also have contemplated similar approaches and compounds and a list is shown in Table 1.

Another approach for marker gene imaging that was unsuccessfully attempted explored the potential of imaging the E.Coli cytosine deaminase (CD) expression with 5-fluoro-cytosine (5FC) (*19*). In our opinion, this approach is doomed to failure because the 5FC is metabolized by the E.Coli cytosine deaminase to 5-fluoro-uracil (5FU) which does not significantly accumulate in the transduced cells because it freely diffuses out.

FUTURE DIRECTIONS

Imaging Multigene Transductions

Since all "therapeutic genes" may not have an appropriate "marker substrate" that can be labeled for imaging (as is the case for HSV1-tk), we are exploring an alternative approach by using a double-gene construct where the "therapeutic gene" is linked to a second "marker gene" (HSV1-tk) by an IRES sequence (*20–22*). We have shown that genes linked by an IRES sequence are expressed in a proportional relationship, such that the magnitude of expression of one gene, the "marker gene," reflects the magnitude of expression of the other gene, the "therapeutic gene". We recently determined the relationship between the expression of GM-CSF and HSV1-tk genes linked by an IRES sequence in transduced murine Lewis Lung carcinoma cells HSV1-tk expression was measured by radiolabeled FIAU accumulation (*10*). Another approach to express two genes in a proportional manner, is based on fusion genes (*23*). Validation of this concept for use in clinical imaging will expand further the utility of HSV1-tk as a "marker gene" in future gene therapy protocols.

Gene-Targeted Radiotherapy

The efficiency of HSV1-tk gene transfer to tumor tissue is relatively low and a significant fraction of the antitumor effect of HSV1-tk/ganciclovir combination gene therapy is based on the "bystander effect." This "bystander effect" is limited to cells that are close to the HSV1-tk transduced cell that is susceptible to ganciclovir treatment. The bystander effect observed with ganciclovir therapy in the nontransduced cells is attributed to ganciclovir monophosphate that is produced by the transduced cells and passed into the surrounding nontransduced cells through the gap junctions. More distantly located cells and, especially, nonproliferating cells are not killed by the bystander effect and are most likely to cause tumor progression or recurrence.

Recently, we developed a new approach for therapy of cancer by merging gene therapy and targeted radiotherapy into a concept that we call gene-targeted radiotherapy (Tjuvajev, *personal communication*, 1997). This new approach uses the HSV1-tk gene transduction of tumor tissue followed by administration of therapeutic doses of radioiodinated FIAU. This approach is aimed not only to improve targeting of the HSV1-tk transduced tumor tissue with radioiodinated FIAU (or other nucleoside analogs) but also to increase the magnitude of the "bystander effect" by ionizing radiation (beta minus) from incorporated ^{131}I-labeled FIAU into the DNA of tumor cells. Indium-125-labeled FIAU could also be used to potentiate the antitumor effect of [^{131}I]-FIAU by more effective elimination of the HSV1-tk transduced tumor cells. It is possible that ^{125}I-labeled FIAU monophosphate could also be shared by the transduced and neighboring nontrans-

duced cells that could result in [^{125}I]-FIAU incorporation into the DNA of nontransduced cells as well. This DNA incorporation should significantly increase the magnitude of bystander effect because each decay of ^{125}I results in (at least) one DNA double-strand break.

A very attractive feature of this approach is that the level of HSV1-tk gene expression after in vivo (or extracorporal) transduction can be imaged with "diagnostic" doses of [^{131}I]- or [^{124}I]-FIAU with gamma camera or PET, respectively. Based on the results of imaging studies, the dosimetry of [^{131}I]- and [^{125}I]-FAIU administration could be estimated as a basis for better treatment planning.

The whole-body radiation exposure from therapeutic doses (100 mCi) is expected to be small and well within acceptable limits based on the results of diagnostic imaging studies of HSV-tk expression in rats. For example, the dosimetry of radioiodinated FIAU may be approximated by radioiodinated IUDR, and estimates of whole-body dose is predicted to be about 0.05 rem/mCi. (*24,25*). Exposure to proliferating tissues after radioiodinated FAIU, (bone marrow and intestinal mucosa) will also be less than that of IUdR that was estimated to be 0.2 rem/mCi. This is because of the substantially lower incorporation of FIAU into nontransduced tissue DNA.

Mutant Receptor Ligands for Radiolabeled Antibodies and Radiolabeled Peptides

Instead of using the natural (wild-type) receptors (as in the currently reviewed paper by Rogers et al. (*5*)) for radio-gene-therapy and/or radio-gene-imaging it is possible to use genetically mutated receptors or truncated receptors. One reason for not using the wild-type receptors is that they remain physiologically active and may respond to naturally present ligands and contribute to tumor growth. Also, because the wild-type receptors are expressed in the tissues of their origin, this creates a limitation from the dosimetry point of view. The later limitation applies also to the dominant-negative receptors (with unmodified binding site). Mutated or truncated receptors devoid these limitations because they are nonfunctional and they are not present in other tissues. Such mutated or truncated receptors can be recognized by a radiolabeled peptide fragment which has a high affinity to the modified receptor but not to the wild-type receptor (or has very low affinity to the wild-type receptor). Alternatively, truncated or heavily mutated (or genetically engineered) "receptors" (any kind of cell membrane incorporating receptor-like protein) can be recognized by a specific radiolabeled antibody that may or may not internalize.

For example, retroviral vectors encoding truncated low-affinity human nerve growth factor receptor (tr-NGRF) have been developed for rapid screening and immuno-magnetic purification of T-cells transduced with this "surface marker" (*26–28*). Using a radiolabeled anti-trNGFR antibody it should be possible to target radioimmunotherapy of transduced tumor tissue.

CONCLUSION

We are the witness to an age in which nature is yielding up her secrets about the role of the genome in human disease. There is reason for optimism, that at least for some patients, what was previously unchangeable bad fortune, can now be fixed through gene therapy. New concepts have emerged that permit gene-selective radiotracer targeting—making it likely that nuclear medicine will soon play its own important role in this grand "triumph over . . . mischance."

Steven M. Larson
Juri Tjuvajev
Ronald Blasberg
Memorial Sloan-Kettering Cancer Center, New York, New York

REFERENCES

1. Fearon ER, Vogelstein B. *A genetic model for colorectal tumorigenesis*. 1990;61:759.
2. Yarbro JW, Bornstein RS, Mastrangelo MJ. Gene therapy. *Semnin Oncol* 1996;23.
3. Krenning EP, Kwekkeboom DJ, Bakker WH. Somatostatin receptor scintigraphy with [In-111-DTPA-D-Phe1]- and [I-125-Tyr3]octreotide: the Rotterdam experience with more than 1000 patients. *Eur J Nucl Med* 1993;20:716–731.
4. Virgolini, I, Raderer M, Angelberger P, et al. Vasoactive intestinal peptide (VIP) receptor imaging for the localization of intestinal adenocarcinomas and carcinoid tumors. *N Engl J Med* 1994;331:1116–1121.
5. Rogers BE, Rosenfeld ME, Khazeli MB, et al. Localization of iodine-125-mIP-Des-Met^{14}-bombesin (7-13)NH_2 in ovarian carcinoma induced to express the gastrin releasing peptide receptor by adenoviral vector-mediated gene transfer. *J Nucl Med* 1997;38:1221–1229.
6. Tjuvajev J, Stockhammer G, Desai R, et al. Imaging gene transfer and expression: paradigm and perspectives. *Proceedings of the 86th Annual Meeting of the American Association for Cancer Research*. Toronto, Canada: AACR; 1995:418.
7. Tjuvajev J, Stockhammer G, Desai R, et al. Imaging gene transfer and expression in vivo. *Cancer Res* 1995;55:6121–6135.
8. Tjuvajev, JG, Finn R, Watanabe K, et al. Noninvasive imaging of herpes virus thymidine kinase gene transfer and expression: a potential method for monitoring clinical gene therapy. *Cancer Res* 1996;56:4087–4095.
9. Tjuvajev J, Finn R, Watanabe K, Joshi R, Beattie B, Blasberg B. Imaging in vivo HSV1-tk gene transfer and expression with [^{124}I]FIAU and PET [Abstract]. *Can Gene Ther* 1996;3:S30.
10. Tjuvajev J, Sharma S, Miller P, et al. Monitoring the expression of therapeutic genes linked to a marker gene by an IRES sequence [Abstract]. *J Neuro-Oncology* 1996;30:P25.
11. KW Morin, EE Knaus, LI Wiebe. HSV-thymidine kinase: molecular target for transfection imaging in gene therapy of cancer [Abstract]. *Antiviral Res* 1995; 26:A304.
12. KW Morin, EE Knaus, LI Wiebe. Noninvasive scintigraphic monitoring of gene expression in HSV-1 thymidine kinase gene therapy model. *Nucl Med Commun* 1997; in press.
13. KW Morin, Elina D Atrazheva, EE Knaus, LI Wiebe. Synthesis and cellular uptake of 2′-substituted analogues (E)-5-(2-[^{125}I]-iodovinyl)-2′-deoxyuridine in tumor cells transduced with the herpes simplex Type 1 thymidine kinase gene. Evaluation as probes for monitoring gene therapy. *J Med Chem* 1997; in press.
14. Srinivasan A, Gambhir SS, Green AL, et al. A PET reporter gene (PRG)/PET reporter gene probe (PRP) technology for repeatedly imaging gene expression in living animals [Abstract]. *J Nucl Med* 1996;37:107P.
15. Goldman S, Monclus M, Cool V, et al. A novel PET tracer for evaluation of gene therapy [Abstract]. *J Nucl Med* 1996;37:53P.
16. Alauddin MM, Conti PS, Lever JR, Mazza SM, Hamzeh FM. Synthesis of F-18 9-[(3-fluoro-1-hydroxy-2-propoxy)-methy]-guanine (FHPG) for in vivo imaging of viral infection and gene therapy with PET. *J Nucl Med* 1996;37:193.
17. Jolly D. Viral vector systems for gene delivery. *Cancer Gene Therapy* 1994;1:51–64.
18. Clinical protocols worldwide. *Cancer Gene Therapy* 1996;3:265–278.
19. Haberkorn U, Oberdorfer F, Gebert J, et al. Monitoring gene therapy with cytosine deaminase: in vitro studies using tritated-5-fluorocytosine. *J Nucl Med* 1996;37: 87–94.
20. Ghattas IR, Sanes JR, Majors JE. The encephalomyocarditis virus internal ribosome entry site allows efficient coexpression of two genes from a recombinant provirus in cultured cells and in embryos. *Mol Cell Biol* 1991;11:5848.
21. Sokolic RA, Sekhsaria S, Sugimoto Y, et al. A bicistronic retrovirus vector containing a picornavirus internal ribosome entry site allows for correction of X-Linked CGD by selection for MDR1 expression. *Blood* 1996;87:42–50.
22. Sugimoto Y, Aksentijevich I, Murray GJ, Brady RO, Pastan I, Gottesman MM. Retroviral coexpression of a multidrug resistance gene (*MDR1*) and human alpha-galactosidase A for gene therapy of Fabry disease. *Hum Gene Ther* 1995;6:905–915.
23. Rogulski KR, Kim JH, Kim SH, Freytag SO. Glioma cells transduced with an Escherichia coli CD/HSV-1 TK fusion gene exhibit enhanced metabolic suicide and radiosensitivity. *Hum Gene Ther* 1997;8:73–85.
24. Macapinlac HA, Kemeny N, Daghighian F, et al. Pilot clinical trial of 5-[I-125]iodo-2′-deoxyuridine in the treatment of colorectal cancer metastatic to the liver. *J Nucl Med* 1996;37:(Suppl):25S–29S,352.
25. Daghighian F, Humm JL, Macapinlac HA, et al. Pharmacokinetics and dosimetry of iodine-125-IUdR in the treatment of colorectal cancer metastatic to liver. *J Nucl Med* 1996;37(suppl):29S–32S.
26. Barba D, Hardin J, Sadelain M, Gage FH. Development of antitumor immunity following thymidine kinase mediated killing of experimental brain tumors. *Proc Natl Acad Sci USA* 1994;91:4348–4352.
27. Sadelain M. Methods for retrovirus-mediated gene transfer into primary T-lymphocytes. In: Robbins P, ed. *Methods in Molecular Medicine, Gene Therapy Protocols*. Totowa, NJ: Humana Press, Inc.;1996:241–248.
28. Frebourg T, Sadelain M, Nug Y-S, Kassel J, Friend SH. Equal transcription of wild-type and mutant p53 using bicistronic vectors results in the wild-type phenotype. *Cancer Res* 1994;54:878–881.