INVITED COMMENTARY

Progress in Gene Therapy: Seeing Is Believing

The past few decades have seen enormous progress in molecular genetics. Techniques such as Southern blotting, Northern blotting, polymerase chain reaction, nucleotide sequencing, chromosome walking, and genetic transfer allow the specific isolation, characterization, and modification of genetic information. Optimists have characterized these developments as "mankind about to determine its destiny," whereas others are afraid that scientists are about to "start playing God."

Developments in molecular genetics have given us insights, at the molecular level, into vital processes in living organisms, such as embryonic development, growth regulation, differentiation, pathogenesis, and carcinogenesis. Insights into the mechanism of pathologic processes, such as developmental disorders and carcinogenesis, have stimulated efforts to develop therapeutic approaches to prevent or correct these processes. Techniques to directly change the genetic information of a cell have raised high expectations of the therapeutic potential of genetic manipulation. In vitro, genes have been introduced successfully into cells, thus changing the genotype and the phenotype of cells. These developments have raised hopes that diseases appearing to be incurable can soon be cured.

Although the basic concept of gene therapy is simple and straightforward, it turned out to be much more complicated in actual practice. In their efforts to transfer genes into animals, investigators quickly realized that numerous technologic problems had to be overcome before gene therapy could be added to the armamentarium of therapeutic approaches of modern medicine. The main obstacles of effective gene therapy are the limited efficiency of the transfer, the limited specificity of the transfer, and the limited duration of the expression of the newly introduced gene.

The design of an effective vehicle to transport the gene into the target cell, the so-called vector, appeared to be of crucial importance. For gene transfer, viral vectors are used. However, most viruses do not allow the incorporation of large genes into their genome. Retroviruses seemed to be attractive candidates because of their ability to stably incorporate their genetic information in the genome of the target cell. Unfortunately, the efficiency of their transfer was low and restricted to dividing cells. Adenoviruses, although efficient vehicles for gene transfer, are easily recognized and neutralized by the immune system. Systematic research during the past decade has revealed new viral constructs for in vivo gene transfer based on lentiviruses, adenovirus-associated viruses, and replication-selective lytic viruses (*1*).

As indicated, the concept of gene therapy has raised high expectations in the medical community and in the general public. However, progress is slower than expected, and in the late 1990s, hope turned into skepticism. The death of 18-y-old Jesse Gelsinger, caused by an unexpectedly vigorous immune response toward an injected adenoviral vector in a gene therapy trial, marked the first crisis in the field (*2*). However, since this sad incident, a few encouraging successes have been reported. A group at the Pasteur Institute in Paris published the first unequivocal results showing that gene therapy can treat patients with the rare immune disease severe combined immunodeficiency-X1 (*3*). Investigators at the Children's Hospital of Philadelphia and at Stanford University proved that hemophilia in patients could be alleviated by intramuscular injection of an adenovirus-associated viral vector containing the factor VIII gene (*4*). A group at the University of Pittsburgh used gene therapy to repair a defect in mice with a type of muscular dystrophy mimicking Duchenne's syndrome (*5*). With a replication-selective lytic viral construct, termed ONYX-015, tumor-selective tissue destruction has been documented in patients with recurrent refractory squamous cell carcinoma of the head and neck (*6,7*).

For further development of gene therapy, it is of the utmost importance that investigators have tools to determine the success of the site-directed gene transfer. In virtually all gene therapy studies, determining whether the transferred gene is expressed in the target cell is important. In that context, investigators need to determine the efficiency, the specificity, and the durability of expression of the therapeutic gene. Reporter gene imaging is a technique that can potentially measure gene expression noninvasively. Imaging reporter gene expression can be a valuable tool to optimize in vivo gene transfer. The study that Yahoubi et al. (*8*) describe in this issue of *The Journal of Nuclear Medicine* marks another step in the development of gene therapy. The radiopharmaceutical in reporter gene imaging is a substrate of the transferred gene, the marker gene. When the marker gene is expressed in the target cell, the gene product (an enzyme) converts the radiopharmaceutical into a metabolite that is selectively trapped within the transfected cell. Alternatively, the transferred gene can induce the expression of a receptor on the cell membrane (i.e., the NaI symporter gene or the dopamine recep-

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tor gene). In the latter form of reporter gene imaging, the radiopharmaceutical is a radiolabeled ligand that specifically binds this receptor (i.e., radioiodide or radiolabeled dopamine, respectively).

At the Memorial Sloan-Kettering Cancer Center, Tjuvajev et al. (*9*) were the first to develop an elegant approach to visualize the expression of the viral enzyme, human simplex virus type-1 thymidine kinase (HSV1-tk). They synthesized radioiodinated 2′fluoro-β-D-arabinofuranosyluracil (FIAU), a radiopharmaceutical that can enter cells by diffusion and by thymidine transporters. FIAU is phosphorylated by HSV1-tk. The phosphorylated FIAU cannot cross the cell membrane and is trapped inside the cell. HSV1-tk not only is a marker gene but also is used as a therapeutic gene: the so-called suicide gene. The growth of HSV1-tk–transfected cells can be effectively arrested with the pro-drugs acyclovir and ganciclovir. These pro-drugs are phosphorylated by HSV1-tk. Subsequently, the phosphorylated pro-drugs are incorporated into the DNA; thus, DNA replication of the transfected cell is arrested.

Imaging of HSV1-tk gene expression can be used in suicide gene therapy to monitor the transfer of the HSV1-tk gene. Thus, HSV1-tk expression imaging can potentially predict the success of treatment with the prodrug. In HSV1-tk gene transfer, the therapeutic gene also acts as the reporter gene. Alternatively, the expression of a gene product of interest can be measured by visualizing the expression of a separate gene using a viral vector containing both genes whose expression is controlled by a single promoter (*10*).

In early studies, FIAU labeled with ^{131}I was used to visualize HSV1-tk gene expression (*9*). Subsequently, FIAU was labeled with ^{124}I to allow PET imaging (*11*). Obviously, PET provides more accurate, quantitative assessment of the expression of the gene than do SPECT techniques.

The group at the Crump Institute for Molecular Imaging at the University of California, Los Angeles, has used ^{18}F-labeled acycloguanosines and uracil analogs to image HSV1-tk expression (*12–14*). On the basis of in vitro and in vivo studies, the ^{18}F-labeled analog of penciclovir, 9-[4-[^{18}F]-fluoro-3-(hydroxymethyl)butyl] guanine ([^{18}F]FHBG)—originally developed by Alauddin et al. (*15*)—was selected for further development (*14*). Yaghoubi et al. (*8*) report on the first use of ^{18}F-labeled analog in 10 healthy volunteers. This ^{18}F-labeled substrate of the HSV1-tk gene product can be synthesized within a reasonable time (95–100 min), at a high specific activity (>37,000 GBq/mmol), and with good radiochemical purity (99%).

The data reported by Yaghoubi et al. (*8*) show that [^{18}F]FHBG was excreted mainly through the kidneys but that a considerable portion was cleared through the hepatobiliary route. Dosimetric analysis of the images indicated that the highest radiation dose was absorbed by the bladder wall (0.1 mGy/MBq) and that the effective dose was acceptable (<0.016 mGy/MBq).

The sensitivity of [^{18}F]FHBG in visualizing cells expressing the HSV1-tk gene has not yet been determined in patients. In mice, cells transfected with the HSV1-tk gene (by intratumoral injection with various viral vectors) could be visualized even in the liver (*16*). Yaghoubi et al. (*8*) indicate that the compound rapidly cleared from virtually all HSV1-tk–negative tissues in the human volunteers. However, whether [^{18}F]FHBG will show HSV1-tk–transfected tissues largely depends on the extent to which [^{18}F]FHBG has accumulated in these tissues. The hepatobiliary excretion of the compound and its excretion in the gut will hamper visualization of HSV1-tk expression in the gut region. The hepatobiliary clearance of a portion of the [^{18}F]FHBG results from the lipophilic character of the compound. Because of its lipophilicity, the compound is able to cross the membrane of the target cell and interact with the HSV1-tk. A marker gene that is expressed on the cell surface, preferably encoding a receptor, will allow the use of a more hydrophilic radiopharmaceutical that is excreted exclusively through the kidneys. Several receptor-ligand targeting systems are available that allow sensitive imaging of receptor-positive cells. Inducing the expression of the NaI symporter gene, as expressed on thyroid follicular cells to induce the accumulation of iodide by the target cell, is an attractive example of such an approach (*17*). Recent studies have shown, however, that an extra gene will be needed to inhibit the subsequent efflux of iodide from the cells (*18*). The gene encoding the somatostatin receptor type 2 (SSTR2) has also been proposed as a marker gene to encode a receptor on the target cell (*19*). Viral vectors with a therapeutic gene and the SSTR2 gene transcribed as 1 messenger RNA (under the control of the same promoter) with an internal ribosomal entry site will allow sensitive imaging of the expression of the therapeutic gene with radiolabeled somatostatin analogs.

In summary, sensitive imaging of reporter gene expression can provide a valuable method to optimize gene therapy. By developing such a technique, nuclear medicine can contribute to the progress in this exciting field. Therefore, we look forward to the first reports on the use of [^{18}F]FHBG in patients whose tumors were transfected with an HSV1-tk–containing vector.

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REFERENCES

1. Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat Med.* 2001;7:33–40.
2. Marshall E. Gene therapy death prompts review of adenovirus vector. *Science.* 1999;286:2244–2245.
3. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science.* 2000;288:669–672.
4. Kay MA, Manno CS, Ragni MV, et al. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat Genet.* 2000;24:257–261.
5. Wang B, Li J, Xiao X. Adeno-associated virus vector carrying human minidystrophin genes effec-

tively ameliorates muscular dystrophy in mdx mouse model. *Proc Natl Acad Sci USA.* 2000;97:13714–13719.
6. Ganly I, Eckhardt SG, Rodriguez GI, et al. A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin Cancer Res.* 2000;6:798–806.
7. Khuri FR, Nemunaitis J, Ganly I, et al. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med.* 2000;6:879–885.
8. 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;42:1225–1234.
9. 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.
10. Gurtu V, Yan G, Zhang G. IRES bicistronic expression vectors for efficient creation of stable mammalian cell lines. *Biochem Biophys Res Commun.* 1996;229:295–298.
11. Tjuvajev JG, Avril N, Oku T, et al. Imaging herpes virus thymidine kinase gene transfer and expression by positron emission tomography. *Cancer Res.* 1998;58:4333–4341.
12. Gambhir SS, Barrio JR, Phelps ME, et al. Imaging adenoviral-directed reporter gene expression in living animals with positron emission tomography. *Proc Natl Acad Sci USA.* 1999;96:2333–2338.
13. Iyer M, Barrio JR, Namavari M, et al. 8-[^{18}F]fluoropenciclovir: an improved reporter probe for imaging HSV1-tk reporter gene expression in vivo using PET. *J Nucl Med.* 2001;42:96–105.
14. Gambhir SS, Herschman HR, Cherry SR, et al. Imaging transgene expression with radionuclide imaging technologies. *Neoplasia.* 2000;2:118–138.
15. Alauddin MM, Shahinian A, Kundu RK, Gordon EM, Conti PS. Evaluation of 9-[(3-^{18}F-1-hydroxy-2-propoxy)methyl]guanine (^{18}F-FHPG) in vitro and in vivo as a probe for PET imaging of gene incorporation and expression in tumors. *Nucl Med Biol.* 1999;26:371–376.
16. Haberkorn U, Henze M, Altmann A, et al. Transfer of the human NaI symporter gene enhances iodide uptake in hepatoma cells. *J Nucl Med.* 2001;42:317–325.
17. Mandell LB, Mandell RZ, Link CJ. Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. *Cancer Res.* 1999;59:661–668.
18. Yu Y, Annala AJ, Barrio JR, et al. Quantification of target gene expression by imaging reporter gene expression in living animals. *Nat Med.* 2000;6:933–937.
19. Zinn KR, Buchsbaum DJ, Chaudhuri TR, Mountz JM, Grizzle WE, Rogers BE. Noninvasive monitoring of gene transfer using a reporter receptor imaged with a high-affinity peptide radiolabeled with ^{99m}Tc or ^{188}Re. *J Nucl Med.* 2000;41:887–895.