Title: METHODS OF DELIVERING GENE THERAPY AGENTS

Abstract: The present invention provides methods for pressure-enhanced delivery of various therapeutic agents, such as gene therapy agents, to the vasculature of a target organ in a mammalian subject. Methods for targeted gene therapy in the mammalian liver as a whole, or in a single hepatic lobe, are disclosed. The disclosed methods rely on minimally invasive catheter-based procedures wherein a target organ is isolated and treated locally with a gene therapy agent. The methods offer more efficient and localized transfection of tissue and are well suited for gene therapy in human subjects.
METHODS OF DELIVERING GENE THERAPY AGENTS

[001] This application claims priority to U.S. Provisional Application Serial No. 60/390,444, filed June 24, 2002, which is incorporated in its entirety by reference.

FIELD OF THE INVENTION

[002] The present invention relates to methods for the administration of therapeutic agents to a target organ of a mammalian subject. More specifically, the invention relates to gene delivery methods whereby a target organ, such as the liver, is isolated and treated employing a minimally invasive, percutaneous transcatheter procedure. The present invention further relates to pressure-enhanced delivery of gene therapy agents directly to the isolated vasculature of a target organ. Methods for targeted gene delivery to the mammalian liver as a whole, or a single hepatic lobe, are disclosed.

BACKGROUND OF THE INVENTION

[003] Gene therapy is the intracellular delivery of exogenous genetic material that corrects an existing defect or provides a new beneficial function to the cells. The liver is an important target organ for gene therapy because of its central role in metabolism and production of serum proteins. There are a large number of known diseases caused by defects in liver-specific gene products or that could benefit from liver production of a secreted protein. Familial hypercholesterolemia, hemophilia, lysosomal storage diseases

[004] Various methods have been developed to deliver exogenous genetic material into cells of the liver. Generally, each method possesses certain drawbacks. For example, ex vivo gene therapy using retroviral vectors requires obtaining hepatocytes from a host, culturing and transfecting the hepatocytes ex vivo and subsequently implanting them back into the host liver (Grossman et al., Nat. Genet. 1994, 6: 335-341). This method is complex and time-consuming, and suffers many drawbacks. Alternatively, delivery of retroviral vectors to the liver directly requires ongoing and increased proliferation of endogenous hepatocytes, which necessitates partial hepatectomy (Ferry et al., Proc. Natl. Acad. Sci. USA 1991, 88: 8377-8381; Kay et al., Hum. Gene Ther. 1992 3, 641-647). When adenoviral gene transfer vectors are injected into the portal vein of a rat, high levels of transgene expression are observed in the liver (Rosefeld et al., Science 1991, 252: 431-434), but such expression is transient and requires repeated injections of the vector. Additionally, when injected in the circulatory system, pre-existing antibodies may quickly neutralize viral vectors. Systemic injections of recombinant adenoviral vectors have shown that a neutralizing host immune response limits the effectiveness of such vectors in repeated injections (Yang et al., Proc. Natl. Acad. Sci. U. S. A. 1994, 91: 4407-4411;
Kozarsky et al., *J. Biol. Chem.* 1994, **269**:13695-13702). Systemic administration of adenoassociated virus (AAV) for transfection of the liver requires delivery of a relatively large number of viral particles (on the order of $10^{11} - 10^{13}$ viral genomes/kg) (Mount et al., *Blood* 2002; **99**: 2670-2676). Production of the necessary number of viral particles required for treatment of human subjects may be difficult to achieve.

[005] Non-viral gene transfer methods, such as injection of "naked" plasmid DNA, have also been described. However, the levels of gene transfer are generally too low to be sufficient for clinical applications (Malone et al., *J. Biol. Chem.* 1994, **269**: 29903-29907; Hickman et al. *Hum. Gene Ther.* 1994, 5:1477-1483). Cationic liposome-mediated gene transfer was found to be much more efficient than transfection with naked plasmid DNA, but the level of gene transfer usually is still not as high as with viral vectors (Liu et al., *J. Biol. Chem.* 1995, **270**: 24864-24870).

[006] Most known gene therapy agents, viral and non-viral, can produce adverse side effects that can restrict their applicability for systemic administration. Therefore, one of the major challenges for gene therapy research has been delivering the agent to a selected site in the body in a highly targeted manner. United States Patent No. 6,265,387 describes a method for transfecting the mouse liver through intravenous injection of a proportionally very large volume of hypertonic solution containing naked plasmid DNA. Although the majority of gene transfer occurs in the liver, other organs such as the spleen, lung, and kidney are also affected, albeit to a much lesser degree. Such an approach is not clinically feasible in the
human due to the relatively high volume of injection required, as well as the indiscriminate transfection of organs other than the liver. To better restrict transfection to the liver and limit the volume of injection in larger species such as the dog, it has been shown previously that high volume hydrodynamic delivery of plasmid DNA into either the vena cava or portal vein can yield high levels of gene transfer if venous outflow from the liver is temporarily interrupted with surgical clamps (Zhang et al., *Hum. Gene Ther.* 1997, **8**: 1763-1772). However, open surgical interruption and a proportionally very high volume of injection, required by this method, increase the risk of the procedure, including risk of cardiodynamic instability and death, making this method clinically unfeasible in human subjects.

[007] Specially designed balloon occlusion catheters have been used to deliver a relatively small volume of a transfection agent to an isolated section of a blood vessel (United States Patent Nos. 5,298,531 and 6,135,976). However, using conventional parameters for injection rate and pressure, methods utilizing such catheters are generally suitable only for transfection of endothelial cells lining the surface of a small section of a vessel lumen. In contrast, the use of such methods for transfection of whole organs, such as the liver, is problematic because capacitance vessels and a dual vascular supply cause rapid losses of injected solution and, consequently, poor transfection efficiency.

[008] Therefore, advancements in hepatic gene therapy in human subjects largely depend on the development of gene delivery methods that are highly
targeted and produce desirable levels of transgene expression in a sustainable fashion.

**BRIEF DESCRIPTION OF FIGURES**

[009] **Figure 1** illustrates a lobar delivery method of hepatic gene therapy utilizing a balloon occlusion balloon catheter introduced through a jugular vein; other transvascular routes, such as a femoral vein, could also be used.

[010] **Figure 2** shows the distribution of gene expression in hepatic parenchymal tissue approximately 24 hours following lobar delivery.

[011] **Figure 3** illustrates the distribution of the gene therapy agent following a high-pressure lobar injection into a single hepatic lobe using the hepatic vein combined with inferior vena cava outflow blockage.

[012] **Figure 4** illustrates targeted whole-organ delivery using a three-catheter approach.

[013] **Figure 5** shows the distribution of gene expression following targeted whole-organ delivery through the vena cava combined with outflow blockage.

[014] **Figure 6** illustrates a double catheter method for whole-organ delivery to the liver through controlled-pressure injection into the vena cava combined with outflow blockage of the liver.

[015] **Figure 7** depicts a catheter system for controlled-pressure targeted whole-organ delivery to the liver through injection into the vena cava combined with outflow blockage of the liver.
[016] **Figure 8** illustrates the placement of a single catheter for targeted whole-organ delivery to the liver through injection into the vena cava combined with outflow blockage of the liver.

[017] **Figure 9** shows representative pressure profiles in the isolated hepatic vasculature between the two occlusion balloons during injection of various volumes (5, 10, and 15 ml/kg of body weight) of DNA-containing solution. The basal vena cava pressures have been subtracted so that the data represent the change in pressure during injection. Pressure was monitored by coupling the injection lumen(s) of one or both of the balloon catheters to the transducer(s) of a blood pressure analyzer.

[018] **Figure 10** shows SEAP expression in the serum of rabbits 24 hours post-transfection of various volumes (5, 10, and 15 ml/kg body weight) of DNA-containing solution. All animals were injected at the rate of 10 ml/sec with the same dose of DNA (2.5 mg/kg pCF1-SEAP with or without 2.0 mg/kg pGZB-sCAT). Addition of pGZB-sCAT had no effect on SEAP expression levels.

**SUMMARY OF THE INVENTION**

[019] The present invention relates to methods by which a depot organ, such as the mammalian liver, is isolated and treated with a therapeutic agent, such as in gene therapy. The methods of the invention employ a minimally invasive, catheter-based procedure. In one method, a balloon-occlusion balloon catheter is engaged proximally in a single hepatic vein and a therapeutic solution is hydrodynamically delivered beyond the inflated
(occluding) balloon via a catheter to the liver parenchyma through the vessels of the thus-isolated target lobe (referred to herein as "lobar delivery"). The rapid rate of injection produces a desired transient increase in pressure within the isolated section of the vasculature. By repositioning the balloon to occlude different vessels, multiple lobes can be treated sequentially during the same procedure. Because treatment is highly localized, various parts of a single organ can be treated in the same procedure with different therapeutic agents that may otherwise be incompatible. Alternatively, with an occluding balloon in the intrahepatic portion of the vena cava blocking venous outflow, an endovascular injection catheter with side holes positioned beyond the balloon in a hepatic vein can be used to treat a given hepatic lobe. By repositioning the endovascular catheter in different vessels, multiple lobes can be treated sequentially during the same procedure.

[020] In another method according to the present invention, venous outflow from the entire organ is temporarily occluded by the placement of balloon catheters in the inferior vena cava both proximal and distal to the hepatic venous outflow, and the gene therapy agent is injected via an endovascular injection catheter in the space between the inflated (occluding) balloons at a rate and volume sufficient to elevate pressure at least transiently and to fill the isolated vascular tree supplying or draining the subtending organ parenchyma (referred to herein as targeted whole-organ delivery). When this method is used to deliver a DNA-containing solution to the isolated liver at adequate pressure, highly effective gene transfer is achieved. In certain
embodiments, the gene therapy solution is hydrodynamically injected at a rate sufficient to result in a pressure increase chosen from the ranges of 10 to 100, 10 to 80, 10 to 50, 20 to 100, 20 to 80, and 20 to 50 mmHg. In further embodiments, the pressure increase is chosen from the ranges of 15 to 100, 15 to 80, 15 to 50, 10 to 30, and 10 to 20 mmHg. In certain embodiments, the pressure increase is at least 15 mmHg. In certain related embodiments, the catheters are rated to withstand injection pressures of at least 300 psi, and preferably 1200 psi.

DETAILED DESCRIPTION OF THE INVENTION

[021] The term “polynucleotide” refers to deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, nucleotide analogs, and single or double stranded polynucleotides. Examples of polynucleotides include, but are not limited to, plasmid DNA or fragments thereof, viral DNA or RNA, anti-sense RNA, etc. The term “plasmid DNA” refers to double stranded DNA that is circular.

[022] The term “transgene” refers to a polynucleotide that is introduced into the cells of a tissue or an organ and is capable of being expressed under appropriate conditions, or otherwise conferring a beneficial property to the cells. A transgene is selected based upon a desired therapeutic outcome. It may encode, for example, hormones, enzymes, receptors, or other proteins of interest. For instance, in the treatment of familial hypercholesterolemia,
one may use a transgene encoding LDL receptor (Kobayashi et al., *J. Biol. Chem.* 271: 6852-6860).

[023] The term “transfection” is used interchangeably with the term “gene transfer” and means the intracellular introduction of a polynucleotide. “Transfection efficiency” refers to the relative amount of the transgene taken up by the cells subjected to transfection. In practice, transfection efficiency is estimated by the amount of the reporter gene product expressed following the transfection procedure.

[024] The term “transfection agent,” used interchangeably with the terms “gene therapy agent” and “therapeutic agent,” refers to any solution, mixture, or other formulation containing a polynucleotide to be delivered intracellularly. A transfection agent usually includes a carrier polynucleotide, termed “expression vector,” also known as “gene delivery vector,” linked to a transgene and, optionally, other compounds that may facilitate the transfer of the polynucleotide across the cell wall. Typically, such compounds reduce the electrostatic charge of the cell surface and the polynucleotide itself, or increase the permeability of the cell wall. Examples include cationic liposomes, calcium phosphate, polylysine, vascular endothelial growth factor (VEGF), etc. Hypertonic solutions, containing, for example, NaCl, sugars, or polyols, can also be used to increase the extracellular osmotic pressure thereby increasing transfection efficiency. The gene therapy solutions may also include enzymes such as proteases and lipases, mild detergents and other compounds that increase permeability of cell membranes. The methods of the invention are not limited to any particular composition of the
transfection agent and can be practiced with any suitable agent so long as it is not toxic to the subject or its toxicity is within acceptable limits.

[025] The term "hydrodynamic injection" refers to an intravascular injection at a rate and volume sufficient to generate supra-systemic pressure within the vascular space and the subtending organ parenchyma. This pressure increase may be transient, as in a systemic injection without outflow blockade, or prolonged, as in a targeted whole-organ injection with outflow blockade. Hydrodynamic injection may be rate- and volume-controlled by a power injector device programmed to deliver a given volume of therapeutic agent in solution at a given rate. A hydrodynamic injection may also be pressure-controlled, by regulating the power injector by a feedback mechanism that monitors the intravascular or intraparenchymal pressure during the injection cycle. Both rate-controlled and pressure-controlled hydrodynamic injections are referred to herein as "controlled-pressure injections". The methods of invention can be practiced with any suitable pressure profile, without regard to rate of rise, peak pressure, or duration so long as the method is not unduly injurious to the subject.

[026] A salient feature in the methods of the present invention is that delivery of a gene therapy agent to isolated segments of an organ’s vasculature is performed under increased pressure in that segment or segments. The temporary pressure increase is achieved by a hydrodynamic injection of the therapeutic solution. This increase in pressure corresponds to the difference in pressure in the isolated segment of the body vasculature immediately prior to injection and the elevated or supra-systemic pressure
during injection. In certain embodiments, the total volume of injection (usually about 10-50% of the volume of a target organ) is hydrodynamically injected at a rate sufficient to result in a pressure increase chosen from the ranges of 10 to 100, 10 to 80 mmHg, 10 to 50, 20 to 100, 20 to 80, and 20 to 50 mmHg. In further embodiments, the pressure increase is chosen from the ranges of 15 to 100, 15 to 80, 15 to 50, 10 to 30, and 10 to 20 mmHg. In certain embodiments, the pressure increase is at least 15 mmHg. In certain related embodiments, the catheters are rated to withstand injection pressures of at least 300 psi, and preferably 1200 psi. Comparisons of transfection efficiencies indicate that hydrodynamic delivery of a therapeutic agent has significant advantages over local application of equivalent or higher levels of pressure externally. The present invention allows highly efficient transfection of a target organ such as the liver with a substantially lower dose of gene therapy agents delivered, as compared to systemic delivery, and decreased exposure of non-target tissues.

[027] In one embodiment of the present invention, a single lobe of the liver is transfected using a controlled-pressure injection. A catheter is advanced, using known interventional and surgical techniques, through the vena cava and into the desired hepatic vein, as depicted in Figure 1. A balloon occlusion balloon catheter is placed within the lumen of a selected hepatic vein following introduction of the catheter through either the jugular or femoral veins. Immediately prior to endovascular transcatheter injection of the transfection agent, a balloon on the catheter is inflated to block venous outflow, thus confining the injected solution to the parenchyma of the isolated target lobe.
[028] In another embodiment of the invention, a single hepatic lobe is transfected under controlled-pressure conditions. A balloon occlusion balloon catheter (as depicted in Figure 3), or an endovascular injection catheter with distal sideholes, is placed within the lumen of a selected hepatic vein. An occluding balloon is placed in the hepatic portion of the inferior vena cava to block hepatic venous outflow. Before transcatheter injection of the transfection agent, the balloon is inflated to block venous outflow, thus confining the injected solution to the parenchyma of the isolated target lobe. Injecting through a catheter with side holes allows a higher rate of injection because it reduces the risk of ballistic injury to the tissues due to greater dispersion of the injected solution.

[029] In another embodiment of the invention, a single lobe is transfected using a hydrodynamic injection. A balloon occlusion balloon catheter is placed within the lumen of a selected hepatic vein. An endovascular injection catheter with sideholes near the tip is advanced through the vena cava into the selected hepatic vein and positioned with the side holes beyond the balloon occlusion balloon. Before endovascular transcatheter injection of the transfection agent, the balloon occlusion balloon is inflated to block venous outflow, thus confining the injected solution to the parenchyma of the isolated target lobe.

[030] In another embodiment of the invention, the transfection agent is delivered to the entire liver with a single hydrodynamic injection. As depicted in Figure 4, the liver is isolated through the use of balloons delivered via two separate dual-lumen catheters, inflated in the inferior vena cava both superior and inferior to the hepatic venous outflow. The transfection agent is then injected through an endovascular catheter with side holes positioned between the balloons and flows in a retrograde
fashion through the hepatic veins to the entire hepatic parenchyma. As depicted in
Figure 6, the endovascular catheter with side holes may be incorporated with one
of the balloon occlusion balloon catheters, reducing the number of catheters that
must be deployed, and eliminating loss of pressure alongside the delivery catheter.
Catheters can be delivered transvascularly using a minimally invasive image-
guided percutaneous technique rather than open surgical cutdown to the vessel
achieve blockade of target organ vascular outflow.

[031] In all embodiments of the invention, the balloons are sized to each patient’s
vessels to assure atraumatic blockage of target-organ vascular outflow during the
procedure. The desired solution is injected at controlled rate and volume into the
isolated vascular space through the catheter. A constricting band may be applied
across the subject’s abdomen over the liver to limit the volume of injected solution
required to generate a given pressure by limiting expansion of the isolated organ
during the injection.

[032] Physical methods such as, for example, electroporation and ultrasound-
mediated sonoporation with or without microbubbles (microbubble booster), may be
used with the methods of this invention to increase transfection efficiency. For
example, if the expression levels obtained by the methods of the invention are
therapeutic, but there is unacceptable risk of tissue injury due to the high
intrahepatic pressure developed during the procedure, the ultrasound treatment
concurrent with transfection may be used in conjunction with catheter-based
localized delivery to effectuate the same therapeutic result using lower volumes of
injection. Standard therapeutic ultrasound conditions (1 MHz) can be used to
enhance the permeabilization of the liver. Ultrasound may be applied
transcutaneously over the liver prior to, concurrently with, or following delivery of the transfection agent. To further increase the effect of ultrasound treatment, the solution of gene therapy agent may be prepared to contain gaseous microbubbles (0.1 to 100 μm in diameter) also known as microbubble booster. Microbubbles are created, for example, by pre-treating the solution with ultrasound under a gaseous atmosphere. To even further increase efficiency of transfection, the liver can also be treated with therapeutic ultrasound prior to delivery of the gene therapy agent.

[033] The methods of the present invention involve the use of a transfection agent in the course of gene therapy, however, the methods apply equally well to therapeutic injections of chemotherapeutic or other pharmaceutical agents, stem cells, or imaging contrast materials where targeted delivery of a diagnostic or therapeutic solution at controlled pressure to an isolated organ is desired.

[034] The following representative examples are intended to illustrate, but not limit, the present invention. While the representative procedures are performed in rabbits, they are successfully performed within parameters clinically feasible in human subjects.

EXAMPLES

[035] In all examples, New Zealand White rabbits were obtained from Millbrook Breeding Lab (Amherst, MA). Catheters and guidewires were obtained from Boston Scientific (Natick, MA) or Cook (Bloomington, IN). The power injector and Optiray 350 contrast medium were obtained from Mallinckrodt (Hazelwood, MO). Pressure in the isolated section of the vasculature was measured through one of the (non-injecting) balloon catheters using a Blood Pressure Analyzer (Micro-Med,
Louisville, KY). Under the conditions described in the examples, an increase of 10-60 mmHg in pressure was achieved for injection rates of 5 to 15 ml/sec.

**Example 1** Lobar Delivery

[036] The jugular vein of a rabbit was accessed through a 2.5 cm paramedian longitudinal incision beginning at the inferior margin of the mandible and extending caudally. A balloon occlusion balloon catheter was advanced through the vena cava and into the desired hepatic vein as depicted in **Figure 1**. The muscle fascia was bluntly dissected to expose the right external jugular vein. A 20-gauge angiocatheter needle was inserted into the jugular vein. Alternatively, an image-guided percutaneous needle, an approach more likely to be employed in humans, can be used to access the vein. Under fluoroscopic guidance, a 0.018-inch Teflon-coated guidewire was placed coaxially through the angiocatheter and guided into a hepatic vein under fluoroscopic guidance. The angiocatheter was exchanged over the guidewire for a 5 French balloon occlusion balloon catheter that was guided selectively into the target hepatic lobar vein. The guide wire was removed and a small amount of non-ionic iodinated contrast agent was injected to confirm proper positioning fluoroscopically. The balloon occlusion balloon was inflated with contrast and occlusion of the selected vessel confirmed by injection of a small volume of contrast. An aliquot (5-15 ml per kilogram of body weight) of the transfection agent containing approximately 2 mg/kg of the pCF1-SEAP plasmid (with or without co-administration of the pGZB-sCAT plasmid), or 7 mg of the pGZB-α galactosidase A (α-gal) plasmid in saline (with or without 7.5-15%
mannitol) was then injected through the endovascular catheter into the isolated lobe at varying rates.


[038] The overall efficiency of transfection was evaluated by measuring the concentration of SEAP in the serum of animals 1 to 3 days post transfection. The rabbit serum was heated to 65°C to denature endogenous alkaline phosphatase and assayed for SEAP activity per manufacturer’s instructions using an alkaline phosphatase reagent from Sigma-Aldrich (St. Louis, MO) and human placental alkaline phosphatase from Calbiochem (LaJolla, CA) as a standard. Serum SEAP levels were in the range of 1-50 μg/ml, as shown in Table 1. These levels are 16 to 800 times lower than the levels achieved in a mouse following high-volume (hydrodynamic) tail vein injection. The efficiency of transfection was dependent on the volume of injection, the rate of injection and the DNA concentration employed.

[039] With a single end-hole catheter maximal injection rates of approximately 5 ml/sec were achievable, with higher rates causing injury to the hepatic vein and subtending parenchyma. With a catheter containing multiple side-holes, injection rates of up to 15 ml/sec were achieved without significant liver injury.

[040] Lobar injection with balloon occlusion of a single lobar vein demonstrates recirculation of the contrast material under fluoroscopic imaging. As observed, the
bolus is initially distributed throughout the parenchyma of the isolated lobe, distal to the point of obstruction. Immediately thereafter, contrast is visualized filling the portal vein in a retrograde fashion, and is quickly but much less densely distributed to the remaining (non-isolated) hepatic parenchyma. In addition, some of the injectate is distributed from the portal vein in a retrograde fashion to the superior mesenteric and splenic veins. Finally, the injected bolus passes to the hepatic veins, and via the vena cava with the systemic venous return to the right atrium. Within a few seconds following the injection, contrast density in the renal cortex is seen indicating that the distribution is now systemic.

[041] The expression of alpha-galactosidase was measured to evaluate the transfection distribution in the liver. The assay was performed according to the procedure described in Ziegler et al., *Hum. Gene Ther.* 1999, 10:1667-1682.

[042] The levels of alpha-galactosidase expression, shown in Figure 2, confirm that redistribution of the injection to the non-isolated portions of the liver occurs. Portal redistribution results in levels of transfection that are an order of magnitude lower than the corresponding levels in the isolated target lobe. However, due to portal redistribution, the level of alpha-galactosidase expression even in the non-isolated liver is still significantly above the level that can be achieved with low-pressure (non-hydrodynamic) systemic injections.
Table 1  Levels of SEAP expression following transfection using lobar delivery

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Injection Volume, ml</th>
<th>Injection Rate, ml/sec</th>
<th>Serum SEAP, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>59a</td>
<td>60</td>
<td>2</td>
<td>6.9</td>
</tr>
<tr>
<td>59b</td>
<td>60</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>74a</td>
<td>25</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>74b</td>
<td>50</td>
<td>5</td>
<td>5.9</td>
</tr>
<tr>
<td>71a</td>
<td>60</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>73a</td>
<td>60</td>
<td>5</td>
<td>4.7</td>
</tr>
<tr>
<td>73b</td>
<td>60</td>
<td>5</td>
<td>6.6</td>
</tr>
<tr>
<td>72</td>
<td>60</td>
<td>15**</td>
<td>1.8</td>
</tr>
<tr>
<td>74c</td>
<td>75</td>
<td>5</td>
<td>21.9</td>
</tr>
<tr>
<td>74d</td>
<td>100</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>71b</td>
<td>60*</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>71c</td>
<td>60*</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>71d</td>
<td>60*</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>73c</td>
<td>80*</td>
<td>5</td>
<td>46.6</td>
</tr>
<tr>
<td>73d</td>
<td>80*</td>
<td>5</td>
<td>26.6</td>
</tr>
</tbody>
</table>

* Two hepatic lobes were sequentially transfected during the procedure.
** Substantial leakage past the occluding balloon was observed.

Example 2  Lobar Delivery with Outflow Blockade

[043] The lobar delivery method, as described in Example 1, restricts high-pressure delivery to a portion of the depot organ distal to the occlusion balloon. To increase pressure in the remainder of the liver during the lobar delivery procedure, outflow blockade of all hepatic veins can be achieved by covering the hepatic venous ostia with a balloon catheter deployed in the hepatic vena cava.

[044] To perform this procedure, the femoral vein was accessed from the medial thigh via a longitudinal skin incision extending inferiorly from the femoral groove. Muscle fascia was bluntly dissected to expose the
neurovascular bundle. The femoral vein was carefully dissected from the
associated artery and nerve. A 1-2 cm segment of the femoral vein was
isolated and ligated distally. A 7 French introducer sheath was inserted into
the femoral vein, proximal to the ligation. A guidewire was advanced into the
inferior vena cava using fluoroscopic guidance. A 5 French catheter with a
14 mm by 4 cm noncompliant balloon was passed through the sheath over
the guidewire into the hepatic portion of the vena cava. The outer diameter
of the sheath is large relative to the rabbit femoral vein, making this
procedure difficult to replicate in the rabbit model without vascular injury.
This complication is not expected in human subjects.

[045] The rabbit was systemically heparinized. The balloon was inflated just prior
to injection of the transfection agent via the balloon occlusion balloon catheter
placed in a hepatic vein. Following inflation of the balloon, a small amount of
radiographic contrast was injected through the introducer sheath to ensure that the
balloon obstructed flow in the inferior vena cava. 70 ml of transfection agent
containing 7 mg of the pCF1-SEAP plasmid and 15% mannitol in saline was then
injected through the endovascular catheter into a single isolated lobe at the rate of
5 ml/sec. Immediately thereafter, the catheters and sheath were withdrawn and
hemostasis was achieved. Dense radiographic contrast, as seen in Figure 3,
enhanced the isolated lobe, while more dilute contrast recirculated in a retrograde
fashion to the remainder of the liver via the portal vein.

[046] The level of serum SEAP was 37 μg/ml as measured by the procedure
described in Example 1. These results demonstrate that lobar delivery with balloon
catheter blockade of hepatic venous outflow is as efficient for gene transfer as
sequential lobar delivery to multiple lobes without outflow blockade (**Table 1**, experiments 71a-d, 73a-d).

**Example 3**  
Lobar Delivery via Portal Vein

[047] Prior investigators have suggested that delivery of transfection agent solution using the liver as a depot organ may also be accomplished via portal vein. Under real-time ultrasound guidance the main portal vein was identified and accessed via a percutaneous transhepatic puncture with an echogenic access needle. Controlled ventilation was useful to limit diaphragmatic motion during the access procedure. Under fluoroscopic control, a small amount of radiographic contrast was injected to confirm needle position. A guidewire was introduced through the needle and the needle exchanged for the sheath of an angiocatheter. Position was again confirmed and 70 ml of transfection agent containing 7 mg of the pCF1-SEAP plasmid and 15% mannitol in saline was then injected into the portal vein. Hemostasis was achieved with digital pressure following removal of the percutaneous access.

[048] The concentrations of serum SEAP achieved in the serum of rabbits treated with this method ranged from 0.2 to 0.8 μg/ml as measured by the procedure described in **Example 1**. Because a much greater proportion of the portal vein is extrahepatic in rabbits than in humans, access using this approach and adequate hemostasis following the procedure are difficult to achieve. For this reason, outflow blockade was not attempted and suprasystemic pressures were not achieved.
during injection. These factors are not expected to be limiting for the equivalent procedure in human subjects.

**Example 4  Targeted Whole-Organ Delivery**

[049] To deliver a gene therapy agent to the entire liver with a single hydrodynamic injection, the liver is isolated through the use of balloons inflated in the inferior vena cava both superior and inferior to the hepatic venous outflow. The transfection agent solution is then injected between the balloons and flows in a retrograde fashion through the hepatic veins to the entire hepatic parenchyma. One version of this method is shown in **Figure 4**. In this version, balloon occlusion balloons are advanced from above through the jugular vein to a position in the inferior vena cava between the right atrium and the most superior hepatic vein, and from below through a femoral vein to a position in the inferior vena cava between the most superior renal vein and the most inferior hepatic vein. A 4 French pigtail catheter with multiple side holes near the tip is advanced through the opposite femoral vein to a position in the inferior vena cava between the two balloon occlusion balloons. The balloons are inflated to isolate the liver immediately prior to the injection of the gene therapy solution via the pigtail catheter.

[050] In an alternate embodiment of this method, the balloon occlusion balloons may be delivered via two separate dual-lumen catheters, as depicted in **Figure 6**, or via a single four-lumen catheter, as shown in **Figure 7** and **Figure 8**.

[051] Injections at sufficient rate and volume to achieve high levels of gene transfection may cause transient cardiodynamic instability (bradycardia and hypotension) that appear to be related to a vasovagal response to stretching of the
vessels and the hepatic capsule during injection, as well as to the rapid volume
loading that accompanies the injection. Pre-treatment of the rabbits with an
anticholinergic agent such as glycopyrrolate just prior to injection prevents this
response. In this example, the transfection agent solution contained 100 μg/ml of
the pGZB-sCAT plasmid, 100 μg/ml of the pCF1-SEAP plasmid and 15% mannitol
in saline solution and was injected at rate of 8-10 ml/sec. Eliminating mannitol from
this solution resulted in increased expression and lower toxicity.

[052] The observed levels of serum SEAP were within between 1/20th and 1/2
those in a mouse following hydrodynamic tail vein injections, as measured by the
procedure described in Example 1. The results of the SEAP measurements are
represented in Table 2.

[053] One day after transfection, core tissue liver samples were obtained from
rabbits post-mortem and analyzed for CAT expression according to the procedure

[054] As shown in Figure 5, this approach results in high levels of tissue
transfection throughout the hepatic parenchyma (compare with Figure 2).
Transgene expression is distributed throughout the hepatic parenchyma, in contrast
to the localized distribution obtained as a result of lobar delivery. Figure 9 shows
representative pressure profiles in the region of the vena cava between the two
occlusion balloons during injection of various volumes (5, 10, and 15 ml per kg of
body weight) of DNA-containing solution. The basal vena cava pressures have
been subtracted so that the data presented represents the change in pressure
during injection. Pressure was monitored by coupling the injection lumen(s) of one
or both of the balloon catheters to the transducer(s) of a blood pressure analyzer.
**Figure 10** shows SEAP expression in the serum of rabbits 24 hours post-transfection of various volumes (5, 10, and 15 ml/kg body weight) of DNA-containing solution. All animals were injected at the rate of 10 ml/sec with the same dose of DNA (2.5 mg/kg pCF1-SEAP with or without 2.0 mg/kg pGZB-sCAT). Addition of pGZB-sCAT had no effect on SEAP expression levels.

**Table 2**  Levels of SEAP expression following targeted whole-organ delivery

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Total Injection Volume, ml</th>
<th>Injection Volume per weight, ml/kg</th>
<th>Injection Rate, ml/sec</th>
<th>Serum SEAP, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>903a</td>
<td>75</td>
<td>10</td>
<td>10</td>
<td>61</td>
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<tr>
<td>903b</td>
<td>75</td>
<td>10</td>
<td>63</td>
<td></td>
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<td>956</td>
<td>80</td>
<td>8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>005a</td>
<td>44</td>
<td>10</td>
<td>2 *</td>
<td></td>
</tr>
<tr>
<td>005b</td>
<td>63</td>
<td>10</td>
<td>401</td>
<td></td>
</tr>
<tr>
<td>958</td>
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<td>10</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>0255a</td>
<td>43</td>
<td>10</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>0255b</td>
<td>45</td>
<td>10</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>0259a</td>
<td>41</td>
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<td>3</td>
<td></td>
</tr>
<tr>
<td>0259b</td>
<td>37</td>
<td>10</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>0260a</td>
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<td>0260b</td>
<td>19</td>
<td>5</td>
<td>0.03</td>
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<tr>
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<td>0263c</td>
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<td>50</td>
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<td>188</td>
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</tr>
</tbody>
</table>

* low expression likely related to low injection volume
Example 5  Abdominal Banding

[055] During studies to assess whole organ delivery using the triple catheter approach, it was noted that in a number of animals, significant loss of contrast was occurring through the portal system. In addition, it was reasoned that the ability of the liver to expand during injection may be limiting the intravascular pressure that could be achieved during injections. In order to limit volume loss through the portal vasculature and to increase intravascular pressures while using reduced volumes, the abdomen was banded in such a way as to reduce the ability of the liver to expand and to apply pressure to the portal vein.

[056] The abdomen of the rabbits was banded with a blood pressure cuff which was inflated just before injection to either 30 or 60 mmHg. As observed by fluoroscopy, this manipulation increased the intensity of contrast in the vasculature while parenchymal staining was reduced. Pressure monitoring indicated that the addition of the blood pressure cuff resulted in significantly higher pressures in the vena cava during injection than observed with non-banded animals using the same injection volume. However, this procedure resulted in significantly lower overall expression levels (Table 3) than observed for animals that were not bound using otherwise identical delivery conditions (10 ml/kg, 10 ml/sec, 2.5 mg/kg pCF1-SEAP). Overall SEAP expression levels animals without banding were 23.22±14.87 μg/ml (n=4, Table 2, exp. # 0255a-b, 0259a-b). Under the same delivery conditions, SEAP expression levels in animals with cuffs inflated to 30 and 60 mmHg were 2.51±3.49 (n=6) and 12.53±15.72 μg/ml (n=6) respectively (Table 3).
[057] The specification is most thoroughly understood in light of the teachings of the references cited within the specification, all of which are hereby incorporated by reference in their entirety. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan recognizes that many other embodiments are encompassed by the claimed invention and that it is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

Table 3 Levels of SEAP expression following targeted whole-organ delivery with abdominal banding

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Cuff Pressure, mmHg</th>
<th>Serum SEAP, µg/ml</th>
</tr>
</thead>
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<tr>
<td>0394a</td>
<td>30</td>
<td>0.31</td>
</tr>
<tr>
<td>0394b</td>
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<td>43.8</td>
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<tr>
<td>0396b</td>
<td>60</td>
<td>4.08</td>
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<tr>
<td>0396c</td>
<td>60</td>
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<td>0397a</td>
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<td>8.58</td>
</tr>
</tbody>
</table>
CLAIMS

What is claimed is:

1. A method for performing gene therapy on a selected organ of a mammalian subject, comprising the steps of:
   (a) placing one or more catheters within the vasculature of the organ; at least one of the catheters having one or more occlusion members;
   (b) isolating a section of the vasculature by occluding flow of fluids with the catheter;
   (c) delivering a gene therapy agent under increased pressure into the isolated section of the vasculature; and
   (d) allowing the gene therapy agent to persist within the isolated section of the vasculature for a period of time sufficient for transfection of a therapeutically effective amount of the agent.

2. The method of claim 1, wherein the catheter is a balloon occlusion balloon catheter.

3. The method of claim 1, wherein the increase of pressure is chosen from the ranges of 10 to 100, 10 to 80, and 10 to 50 mmHg.

4. The method of claim 1, wherein the increase of pressure is chosen from the ranges of 20 to 100, 20 to 80, and 20 to 50 mmHg.

5. The method of claim 1, wherein the gene therapy agent is delivered via an endovascular catheter.

6. The method of claim 5, wherein the wall strength of the catheter is rated to withstand injection pressures of at least 300 psi.
7. The method of claim 6, wherein the wall strength of catheter is rated to withstand injection pressures of at least 1200 psi.

8. The method of claim 1, wherein the gene therapy agent is delivered via a percutaneous needle.

9. The method of claim 1, wherein the gene therapy agent comprises a nucleic acid.

10. The method of claim 9, wherein the nucleic acid is plasmid DNA.

11. Method of claim 10, wherein the nucleic acid is a viral expression vector.

12. The method of claim 1, wherein the organ is the liver.

13. The method of claim 12, wherein the section of the vasculature comprises an intrahepatic portion of the inferior vena cava.

14. The method of claim 12, wherein the section of the vasculature comprises an intrahepatic portion of the portal vein.

15. The method of claim 12, wherein the section of the vasculature comprises a portion of the hepatic artery.

16. The method of claim 1, wherein the organ is a single hepatic lobe.

17. The method of claim 16, wherein the section of the vasculature comprises a portion of a hepatic vein.

18. The method of claim 1, further comprising the step of constricting vascular and organ expansion with a band about the body...
overlying the isolated target organ concurrent with delivery of the gene therapy agent.

19. The method of claim 1, further comprising the step of treating the target organ with therapeutic ultrasound prior to, or concurrently with delivery of the gene therapy agent.

20. The method of claim 1, wherein the gene therapy agent comprises microbubble booster.

21. The method of claim 1, further comprising the step of delivering a membrane-permeabilizing compound to the isolated section of the vasculature.

22. The method of claim 1, further comprising the step of administering an anticholinergic agent to the mammalian subject.

23. The method of claim 1, wherein the increase of pressure is chosen from the ranges of 15 to 100, 15 to 80, 15 to 50, 10 to 30, and 10 to 20 mmHg.

24. The method of claim 1, wherein the increase of pressure is at least 15 mmHg.
FIG. 4
8ml/sec, 18ml/kg
Cat: ng/100ng tissue
Ave: 444ng/100mg

01-0956Rlv: #8

FIG. 5
FIG. 7
FIG. 8
FIG. 9
Fig. 10
A. CLASSIFICATION OF SUBJECT MATTER
   IPC(7) : C12N 15/63, 15/85, 15/87; A01N 43/04; A61K 31/70
   US CL : 435/455;514/44
According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED
   Minimum documentation searched (classification system followed by classification symbols)
   U.S. : 435/455;514/44

   Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
   Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
   Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>US 6,135,976 A (TACHIBANA et al.) 24 October 2000(24.10.2000), see entire document.</td>
<td>1, 2, 9, 10, 11, 19, 20</td>
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<td>12,21,22</td>
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</tbody>
</table>

☐ Further documents are listed in the continuation of Box C.  ☐ See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search: 01 October 2003 (01.10.2003)

Date of mailing of the international search report: 05 NOV 2003

Name and mailing address of the ISA/US
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Authorized officer
Brian Whiteman
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)
Continuation of B. FIELDS SEARCHED Item 3:
WEST2.1, STN
search terms: catheter, occlusion, gene therapy, hepatic artery, liver, organ, balloon occlusion, lobar delivery, hepatic delivery, outflow blockage, hydrodynamic injection, ultrasound, anticholinergic, portal vein, retrograde