Abstract: Inactivation of FHIT, a tumor suppressor gene, is found in a large fraction of cells that are either premalignant or malignant. The present invention relates to gene therapy wherein the FHIT gene is administered, either in vivo or ex vivo, to a patient to inhibit tumor development. The gene therapy methods of the present invention will be used in the treatment of disorders involving an overproliferation of cells. Further, the gene therapy methods of the present invention are useful in protecting a patient that is predisposed to developing a cancer.
**FHIT GENE THERAPY PREVENTS TUMOR DEVELOPMENT IN FHIT-DEFICIENT MICE**

**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims priority to U.S. Provisional Patent Application No. 60/268,090 filed February 12, 2001.

**GOVERNMENT RIGHTS IN THE INVENTION**

This invention was made with government support under grants CCSG P30-CA56036 and PO1-77738 awarded by the USPHS and F32 CA86429-01 awarded by the National Institute of Health. The government has certain rights in the invention.

**FIELD OF THE INVENTION**

The present invention relates to the fields of molecular biology and gene therapy, and to a method of treating or preventing cancer and, more particularly, to the inhibition of tumor development by oral gene transfer using viral vectors expressing the *FHIT* gene.

**BACKGROUND**

Human chromosomal fragile sites map to chromosome bands that are non-randomly altered by translocations or deletions in human neoplasms. (Yunis, J.J. & Soreng, A.L., *Science* 226: 1199-204, 1984). The recombinant nature of fragile sites, possibly enhanced by environmental carcinogens, has been linked to altered expression of oncogenes or tumor suppressor genes at fragile sites. (reviewed in Huebner, K., et al., *Annu Rev Genet* 32: 7-31, 1998). This implies that alteration of expression of genes at fragile sites could trigger clonal expansion of preneoplastic and
neoplastic cells.

FHIT, spanning the most inducible human common fragile site, FRA3B, at chromosome 3p14.2, is thus far the only example of a frequently altered gene at a constitutive fragile region and shows hallmarks of a tumor suppressor gene. (Ohta, M., et al., *Cell* 84: 587-97, 1996). The FHIT gene is altered by deletion in a large fraction of many types of cancer, including, but not limited to: lung, breast, head and neck, cervical, bladder, esophageal, gastric and pancreatic cancer. (Huebner, K., et al., *Annu Rev Genet* 32: 7-31, 1998; Huebner, K., et al., *Advances in Oncology* 15: 3-10, 1999). FHIT is also interrupted by a translocation in a family with predisposition to the development of renal carcinomas. Fhit protein is lost or reduced in the majority of these cancers, in a large fraction of other cancer types, and preneoplastic lesions in the esophagus and lung. (Huebner, K., et al., *Advances in Oncology* 15: 3-10, 1999).


To further clarify the role of Fhit protein in cancer development, one Fhit allele was inactivated in mouse embryonic stem cells and established Fhit <sup>−/−</sup> heterozygous and Fhit <sup>−/−</sup> homozygous mice. It has been previously demonstrated that Fhit <sup>−/−</sup> mice are susceptible to carcinogen-induced tumor development in the esophagus and forestomach. 100% of the heterozygous mice (Fhit <sup>−/+</sup>) developed multiple tumors in the forestomach and at the squamocolumnar junction (SCJ) after exposure to the carcinogen N- nitrosomethylbenzylamine (NMBA), compared to 25% of mice with intact Fhit alleles (Fhit <sup>+/+</sup> mice). (Fong, L.Y., et al., *Proc Natl Acad Sci U S A* 97: 4742-7, 2000). Fhit <sup>−−</sup> mice are even more sensitive to carcinogen than Fhit <sup>+−</sup> mice and both heterozygous and homozygous knock-out mice exhibit increased frequencies of spontaneous tumors, implying that Fhit knock-out mice will serve as
useful models for tumor treatment and prevention. NMBA induces morphologically similar esophageal lesions in rodents and human. (Stinson, S.F., et al., J Natl Cancer Inst 61: 1471-5, 1978). In analogy to the human distal esophagus, the mouse forestomach has an epithelial lining. The mouse SCJ, the transition zone between epithelial and glandular tissue, corresponds to the human esophago-gastric junction. These structures are commonly used as a model system to study conditions in the distal esophagus in man, as both of these regions have a predilection to cancer development. Furthermore, the incidence of cancer in the distal esophagus is rising. (Blot, W.J., et al., Jama 265: 1287-9, 1991).

ABBREVIATIONS

“FHT” means “Fragile Histidine Triade Gene”
“SCJ” means “squamocolumnar junction”
“NMBA” means “N-nitrosomethylbenzylamine”
“GFP” means “green fluorescent protein”
“AAV” means “Adenoassociated Virus”
“Ad” means “Adenovirus”
“FHL” means “focal hyperplastic lesions”

DEFINITIONS

“therapeutic agent” is any composition containing a nucleic acid encoding the \textit{FHT} gene, or derivative thereof.

SUMMARY OF THE INVENTION

It is an object of the present invention to treat a disorder involving a premalignant or malignant condition in a mammal by the method of \textit{in vivo} gene therapy. A therapeutically effective amount of a viral vector containing a \textit{FHT} gene, or
derivative thereof, is administered to the mammal. A Fhit protein, or derivative thereof, is expressed by the FHit gene, or derivative thereof, in the mammal, thereby inhibiting tumor development. In one embodiment the viral vector is at least one of the group of a recombinant adenovirus or recombinant adeno-associated virus. In another embodiment the viral vector integrates into the cell genome. In another embodiment the viral vector remains episomal. In one embodiment of the present invention the method of gene therapy is used to treat a disorder involving a premalignant or malignant condition in a human.

It is a further object of the present invention to treat a disorder involving a premalignant or malignant condition in a mammal by the method of ex vivo gene therapy. The FHit gene is transferred to cells in tissue culture and cells that have taken up and express the Fhit protein, or derivative thereof, encoded by the FHit gene, or derivative thereof, is isolated. A therapeutically effective amount of these cells are administered to the mammal, thereby inhibiting tumor development. In one embodiment of the invention the mammal is a human. In one embodiment the ex vivo gene therapy results in the FHit gene being integrated into cell genome. In another embodiment the FHit gene remains episomal.

It is an object of the present invention to treat a predisposition to a disorder involving a premalignant or malignant condition in a mammal by the method of in vivo gene therapy. A therapeutically effective amount of a viral vector containing a FHit gene, or derivative thereof, is administered to the mammal wherein the Fhit protein, or derivative thereof, encoded by the FHit gene, or derivative thereof, is expressed, thereby protecting against induction of tumor development. In one embodiment the viral vector is at least one of the group of a recombinant adenovirus or recombinant adeno-associated virus. In another embodiment the viral vector integrates into cell genome. In another embodiment the viral vector remains episomal. In one embodiment of the method of in vivo gene therapy the mammal is a human.

It is an object of the present invention to treat a predisposition to a disorder involving a premalignant or malignant condition in a mammal by the method of transferring a FHit gene to cells in tissue culture. These transduced cells which have taken up and express a Fhit protein, or derivative thereof, encoded by the FHit gene, or derivative thereof, are isolated. A therapeutically effective amount of the transduced cells are administered to the mammal, thereby protecting against induction
of tumor development. In one embodiment the mammal is a human. In a further embodiment the \textit{FHIT} gene is integrated into the cell genome. In another embodiment the said \textit{FHIT} gene remains episomal.

The present invention further relates to an adenovirus vector expressing a Fhit protein, or derivative thereof, encoded by the \textit{FHIT} gene, or derivative thereof. The invention also relates to an adeno-associated virus vector expressing a Fhit protein, or derivative thereof, encoded by the \textit{FHIT} gene, or derivative thereof.

**DESCRIPTION OF THE DRAWINGS**

**Figure 1.** Transgene expression with control vectors in normal murine tissue. (a) section of glandular stomach with β-gal staining 3 days after inoculation with Ad-lacZ. (b) Confocal microscopy image of glandular stomach 14 days after AAV-GFP inoculation. GFP immunohistochemistry of the forestomach region 3 days (c) and 14 days (d) after AAV-GFP infection. Brown chromogen in section d indicates presence of GFP.

**Figure 2.** Fhit expression in murine forestomach epithelium 10 weeks after virus administration (magnification x 200). Fhit immunohistochemistry (infra). (a) Control. (b) AAV-FHT. (c) Ad-FHT. (d) murine lung tissue of AAV-FHIT and Ad-FHT treated mouse.

**Figure 3.** Gross anatomy and histopathology of murine forestomach after FHIT-gene therapy. Typical aspects of NMBA-induced pathology in the control group (a-c) are compared with the 3 treatment groups: AAV-FHIT (d-f), Ad-FHIT (g-i) and the combined treatment group Ad-FHIT with AAV-FHIT (j-l). a,d,g,j. show gross anatomy of the forestomach and SCJ (magnification x 5). b, (magnification x 50) and e,h,k. (magnification x 200) are H&E stained forestomach sections. c, (magnification x 50) and f,i,l. (magnification x 200) depict PCNA immunohistochemistry in forestomach sections adjacent to the corresponding H&E sections. PCNA immunohistochemistry shows abundant, intensely stained cells in S-phase in a control forestomach showing a papilloma with hyperplastic epithelium (b,c). In AAV-FHIT
(e,f), Ad-FHIT (h,i) and the combined treatment group (k,l), PCNA-positive cells are found mostly in basal cells of the near normal epithelium.

**DESCRIPTION OF THE INVENTION**

*Methods*

*Construction of the recombinant Vectors*

cDNAs for green fluorescent protein (GFP) and lacZ are obtained from expression vectors (Clontech). Full-length *FHIT* cDNA is isolated from human normal placental tissue by reverse transcription (RT)-PCR strategy. (Ohta, M., et al., *Cell* 84: 587-97, 1996).

*Adenovirus (Ad)*

The recombinant adenoviral vector is constructed as described previously. (Ishii, H., et al., *Cancer Res*: 2000). In summary, the cDNAs are ligated into an adenoviral backbone vector DNA (Quantum). The adenoviral vector is transfected with human fetal kidney 293 cells (Microbix) with plaque isolation and vector purification after homologous recombination in 293 cells.

*Adenoassociated virus (AAV)*

For the AAV-GFP plasmid, the *GFP* cDNA is linked to the promoter EF and is cloned into the *KpnI* and *HindIII* restriction sites of the multiple cloning site (MCS) of pAM/pL-WPRE-BGH poly A, creating pAM/pL-EF-GFP-WPRE-BGH poly A. For the AAV-FHIT plasmid, the *FHIT* gene fragment is cloned into the Bam HI and HindIII sites of pAM/pL-EF-GFP-WPRE-BGH polyA, replacing *GFP* with *FHIT* to generate pAM/pL-EF-FHIT-WPRE-BGH polyA. The packaging plasmid pDG is co-transfected with the corresponding vector plasmids to generate recombinant AAV-GFP and AAV-FHIT. Virus purification and titration is performed as described previously. (Wolff, G., et al., *J Virol* 71: 624-9, 1997).

*Transgene Expression*

For Ad-LacZ 100 μl of virus (10^{11} plaque forming units/ml) and for AAV-
GFP 100 μl of virus (10^{11} VP/ml) is administered via an orogastric tube into the stomachs of a group of healthy mice (n=6). At 3, 7 and 14 days post viral administration mice are overdosed with pentobarbital and perfused transcardially with saline followed by 2% paraformaldehyde containing 2 mM MgCl₂ and 1.25 mM EGTA in 0.1 M phosphate buffer (pH 8.0) to inhibit endogenous β-galactosidase. (Khuri, F.R., et al., Nat Med 6: 879-85, 2000). The esophagus and stomach is then fixed briefly before cryoprotection in a 30% sucrose solution in PBS. Sections 12 μm in thickness are cut on a cryostat and thaw-mounted onto slides. Sections are immersed briefly in 4% paraformaldehyde, washed extensively with PBS and immersed in a solution containing 1mg/ml X-gal, 2mM MgCl₂, 50 mM K₃Fe(CN)₆ and 50 mM K₄Fe(CN)₆ in PBS overnight at 37⁰C.

**Carcinogenicity Study**

(C57BL/6J x 129/SvJ) F1 mice (B6129F1s) that are Fhit⁻⁻ are produced in the Kimmel Cancer Center (KCC) animal facility. 36 Fhit⁻⁻ mice (20–32 wk) are given six intragastric doses of NMBA (Ash Stevens, Detroit, MI) over the course of 3 weeks at 2 mg/kg body weight. At 4 weeks, one group of 8 animals receives a single dose of Ad-FHIT (10^{11} plaque forming units/ml), a second group of 8 animals receives a single dose of AAV-FHIT (10^{11} vp/ml, 100 μl) and the third treatment group receives the same dose of Ad5-FHIT and AAV-FHIT combined, necessitating a double oral volume. 12 control animals do not receive any recombinant virus. All mice are killed 13 weeks after the initial NMBA dose.

**Immunohistochemistry**

After antigen retrieval, endogenous peroxidase is inhibited with 3% hydrogen peroxide, and nonspecific binding sites are blocked with normal goat serum. Slides are incubated with primary rabbit anti-human Fhit antibody against the C-terminus of the human Fhit protein. (1:1,000 dilution, overnight, Zymed, South San Francisco, CA), followed by incubation with biotinylated goat anti-rabbit antibody. Slides are then incubated with streptavidin horseradish peroxidase (Dako, Carpinteria; 1:1,000 dilution). For PCNA mouse monoclonal antibody (1:500, Santa Cruz, Santa Cruz, CA) is used.
**Gene Therapy**

In a specific embodiment, nucleic acids comprising a sequence encoding a Fhit protein or functional derivative thereof, are administered to promote Fhit function, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. (Vile, R.G., et al., *Gene Therapy* 7:2-8, 2000). The subject, or patient, is preferably an animal, including, but not limited to, animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human. The subject can be a fetus, child, or adult. In a specific embodiment, a non-human mammal is the subject.

A FHit polynucleotide is used in the treatment of various disease states associated with chromosome 3p14.2 abnormalities, such as cancers, and/or decreased expression of wild-type FHIT RNA or protein. By introducing FHit gene sequences into cells, gene therapy is used to treat conditions associated with under-expression of functional FHIT RNA or protein. Accordingly, the present invention provides a method for treating a disease state associated with a chromosome 3p14.2 abnormality in mammal suffering from a disease state associated with a chromosome 3p14.2 abnormality comprising administering a therapeutically effective amount of a nucleic acid encoding a functional Fhit protein to a mammal suffering from a disease state associated with a chromosome 3p14.2 abnormality. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect by promoting Fhit function, thereby, e.g., inhibiting tumor or cancer appearance or progression.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods, which are by no means meant to limit the present invention, are described below.

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In a specific embodiment, the nucleic acid is directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (see U.S. Pat. No. 4,980,286) (see infra), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432, 1987) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated Apr. 16, 1992 (Wu et al.); WO 92/22635 dated Dec. 23, 1992 (Wilson et al.); WO92/20316 dated Nov. 26, 1992 (Findeis et al.); WO93/14188 dated Jul. 22, 1993 (Clarke et al.), WO 93/20221 dated Oct. 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination. (Koller & Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935, 1989; Zijlstra et al., Nature 342:435-438, 1989).

In a preferred aspect, the therapeutic agent comprises a FHIT nucleic acid that is part of an expression vector that expresses a Fhit protein or fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the FHIT coding region, the promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the FHIT coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the FHIT nucleic acid. (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935,

Delivery of the nucleic acid into a patient is either direct, in which case the
patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or
indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then
transplanted into the patient. These two approaches are known, respectively, as *in vivo*
or *ex vivo* gene therapy.

*Administration of vectors expressing FHIT*

In a specific embodiment, the nucleic acid is directly administered *in vivo*,
where it is expressed to produce the encoded product. This is accomplished by
administration of the *FHIT* expressing vectors of the present invention (*infra*). Two
viral categories of viral vectors are used: those that integrate into the cells genome
(including, but not limited to, adeno-associated viral vectors, retroviral vectors, and
lentiviral vectors, *infra*) and those that remain episomal, i.e. non-integrating
(including, but not limited to, adenoviral vectors, *infra*). The integrating viral vectors
allow for the sustained expression of the deficient gene product, while the non-
integrating (episomal) expression vectors allow for a transient transgene expression.

Different routes of administration for *in vivo* therapy include, but are not limited to,
parenteral (intravenous) administration, subcutaneous administration and oral
administration. The viral vector that contains the FHIT nucleic acid is used. The
FHIT nucleic acid to be used in gene therapy is cloned into the vector, which
facilitates delivery of the gene into a patient (*infra*).

Adenoviruses are viral vectors that are used in the gene therapy of the present
invention. Adenoviruses are especially attractive vehicles for delivering genes to
respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they
cause a mild disease. Other targets for adenovirus-based delivery systems are liver,
the central nervous system, endothelial cells, and muscle. Adenoviruses have the
advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson,
*Current Opinion in Genetics and Development* 3:499-503, 1993, present a review of
adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10, 1994,
demonstrated the use of adenovirus vectors to transfer genes to the respiratory
epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene
therapy can be found in Rosenfeld et al., *Science* 252:431-434, 1991; Rosenfeld et al.,

Adeno-associated virus (AAV) is also used in the gene therapy of the present invention (*infra*) (Monahan, P.E., and Samulski, R.J. *Gene Ther* 7, 24-30, 2000). AAV viruses have specific features that render them attractive for human applications. Epidemiological studies indicate that 90% of the human population has been exposed to AAV with no known associated pathologies. In addition to the non-pathogenic nature, other major advantages of AAV include: a) infection of non-dividing cells, b) persistent gene expression through integration into the cellular chromosomes or by conversion into double-stranded episomal forms, c) delivery of the therapeutic gene without co-transfer of any viral genes, and d) natural tropism for epithelial cells. (During, M.J., et al., *Science* 287: 1453-60, 2000; During M.J., et al., *Nat. Med.* 4: 1131-5, 1998; Monahan, P.E. and Samulski, R.J., *Gene Ther.* 7: 24-30, 2000). This allows the transgene to persist in the target tissue without the AAV vector inducing an inflammatory response against the transduced cells. Experiments with recombinant AAV-mediated gene transfer into skeletal muscles and other tissues, for example kidney (Lipkowitz, M.S., et al., *J. Am. Soc. Nephrol.* 10: 1908-15, 1999), have shown persistent transgene expression with no inflammatory responses in animal models and primates. (Xio, X., et al., *J. Virol.* 70: 8098-108, 1996; Fischer, K.J., et al., *Nat Med.* 3: 306-12, 1997; Clark, K.R., et al., *Hum Gene Ther.* 8: 659-69, 1997). While the difficulty of high-titer recombinant AAV (rAAV) vector production has overshadowed its potential benefits, recent advances in the production of high-titer purified rAAV vector stocks have made the transition to human clinical trials a reality. (Monahan, P.E. and Samulski, R.J. *Gene Ther* 7: 24-30, 2000).

The present invention is not limited to the Adenovirus and Adeno-associated virus vectors, other viral vectors that contain the FHIT nucleic acid can also be used. Other viral vectors include, for example retroviral vectors. (see Miller et al., *Meth. Enzymol.* 217:581-599, 1993). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The FHIT nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302, 1994, which describes the use of a retroviral vector to deliver the mdrl gene to

Additional viral vectors that are also useful in the gene therapy of the present invention include, but are not limited to, lentiviral vectors (Pfeifer, A., et al., *Proc Natl Acad Sci U S A* 97, 12227-32, 2000; Klimatcheva, et al., *Front Biosci* 4, D481-96, 1999), modified adenoviral vectors (for example, a modification wherein a surface capsid protein(s) is absent so as to prevent any immune response against the adenoviral vector while still allowing the virus to enter the cell (Zhang, W. W., *Cancer Gene Ther* 6, 113-38, 1999), and replication competent adenoviral vectors that are tumor specific, such as for example reoviruses. (Khuri, F. R., et al., *Nat Med* 6, 879-85, 2000; Heise, C. C., et al., *Cancer Gene Ther* 6, 499-504, 1999; Ries, S. J., et al., *Nat Med* 6, 1128-33, 2000; Coffey, et al., *Science* 282: 1332-4, 1998).

*Ex vivo* FHIT gene therapy

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods, including, but not limited to, electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Further, a chimeric approach using both viral vector with transfecting agent, for example, but not limited to, lipofection, electroporation, calcium phosphate mediated tranfection will also allow for the transfer of a gene into cells. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector.
containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618, 1993; Cohen et al., *Meth. Enzymol.* 217:618-644, 1993; Cline, *Pharmac. Ther.* 29:69-92, 1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique provides for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid are introduced for purposes of gene therapy encompass any desired, available cell type, and include, but are not limited to, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In the embodiment in which recombinant cells are used in gene therapy, a FHIT nucleic acid is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention. Such stem cells include but are not limited to hematopoietic stem cells (HSC), stem cells of epithelial tissues.
such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (PCT Publication WO 94/08598, dated Apr. 28, 1994), and neural stem cells. (Stemple and Anderson, *Cell* 71:973-985, 1992).

Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures. (Rheinwald, *Meth. Cell Bio.* 21A:229, 1980). In stratified epithelial tissue such as the skin, renewal occurs by mitosis of stem cells within the germinal layer, the layer closest to the basal lamina. Stem cells within the lining of the gut provide for a rapid renewal rate of this tissue. ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture. (Rheinwald, *Meth. Cell Bio.* 21A:229, 1980; Pittelkow and Scott, *Mayo Clinic Proc.* 61:771, 1986). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (e.g., irradiation, drug or antibody administration to promote moderate immunosuppression) can also be used.

*Treatment and prevention of disorders involving overproliferation of cells*

In a specific embodiment, digestive tract tumors are treated or prevented, including, but not limited to, esophageal, stomach, pancreas, colon, and colorectal cancers. In another specific embodiment, airway cancers such as lung cancers (e.g., small cell lung carcinoma) and nasopharyngeal carcinoma are treated or prevented. In yet other specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the head, neck, cervix, kidney, stomach, skin, ovary, bladder, breast, colon, lung, or uterus. In other specific embodiments, sarcoma, or leukemia is treated or prevented. In another particular embodiment, osteosarcoma or renal cell carcinoma is treated or prevented.

The therapeutic agents of the invention are also administered to treat premalignant conditions and to prevent progression to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred. (For review of such abnormal growth conditions, see Robbins and Angell,

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Some types of metaplasia are forerunners of cancer, for example metaplasia in the distal esophagus is considered a pre-cancerous lesion. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

In a preferred embodiment of the invention, a patient in whose DNA is detected a mutation (translocation, deletion, insertion, substitution/point mutation or any alteration in the FHIT nucleic acid sequence) in the FHIT gene, particularly a deletion, and most particularly a homozygous mutation, is thereby determined to have a predisposition to cancer and is treated by administration of a nucleic acid encoding the FHIT gene (gene therapy).

Alternatively, or in addition to, the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed in vivo or displayed in vitro by a cell sample from a patient, indicates the desirability of prophylactic/therapeutic administration of the therapeutic agent of the present invention. Such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc.
Therapeutic/prophylactic methods

The invention provides methods of treatment and prophylaxis by administration to a subject of an effective amount of the therapeutic agent, i.e., a FHIT nucleic acid. In a preferred aspect, the therapeutic agent is substantially purified. The subject is preferably an animal, including, but not limited to, animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human. The subject can be a fetus, child, or adult. In a specific embodiment, a non-human mammal is the subject.

Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds are administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents, such as, for example, chemotherapeutic agents (cisplatin, 5-florouracil, alkylating agents etc.) which are known to enhance the efficiency of transgene delivery. (Khuri, et.al., Nat Med. 6: 879-85, 2000; Alexander, et.al., J Virol. 68: 8282-7, 1994). Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.
Therapeutic/prophylactic compositions

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of the therapeutic agent, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes, but is not limited, to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline.

Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The amount of the therapeutic agents of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and is determined by standard clinical techniques. In addition, in vitro assays may be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of
administration, and the seriousness of the disease or disorder, and is decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 µg/kg body weight to 1 mg/kg body weight. Effective doses are extrapolated from dose-response curves derived from in vitro or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

**Results**

To examine the therapeutic approach two viral gene delivery systems are used for the FHIT gene to determine if FHIT gene delivery to the esophagus and forestomach will prevent tumor formation in Fhit+/- mice after carcinogen exposure. Adeno- (Ad) and adenoassociated viral vectors (AAV) are constructed for human FHIT, for lacZ encoding β-galactosidase (β-gal) and for green fluorescent protein (GFP). The AAV-FHIT and Ad-FHIT are tested in vitro and Fhit bioactivity conferred by the vectors is assessed by in vitro induction of apoptosis and in vivo suppression of tumor growth in nude mice, as described previously. (Ishii, H., et al., Cancer Res: 2000).

The stability of infection in murine esophageal and gastric tissue and levels of transgene expression for both viral vectors are investigated by using the control vectors, Ad-LacZ and AAV-GFP. Comparable doses of recombinant Ad virus expressing β-galactosidase (Ad-lacZ) and AAV virus expressing GFP (AAV-GFP) are administered via an intragastric tube, into the stomachs of a group of 6 healthy mice. Mice are sacrificed at 3, 7 and 14 days after viral administration with cryopreservation of esophagus and forestomach. As expected, 5-bromo-4-chloro-3-indolyl-D-galactopyranoside (X-Gal) staining of mice injected with Ad-lacZ reveals blue nuclei in the epithelial layers of the forestomach 3 days post administration (Figure 1). After AAV-GFP administration, GFP immunohistochemistry fails to detect transgene expression at 3 days, with clear transgene expression 2 weeks after viral administration in lamina propria and epithelial cell layers of esophagus and forestomach, confirmed by confocal microscopy imaging of expression in
approximately 80% of the cells. Microscopic sections do not reveal any cytopathic effect attributable to the Ad-lacZ or AAV-GFP viral vectors (Figure 1).


Fhit+/− mice (age 20 to 32 weeks) receive 6 doses of NMBA through oral gavage at intervals of 3 or 4 days. After 4 weeks, mice are divided into 4 groups: one group of 12 mice remained untreated, two groups of 8 mice each receive a single intra-gastric administration of comparable doses of Ad-FHIT or AAV-FHIT. An additional group of 8 mice receive a combined administration of AAV-FHIT and Ad-FHIT. During the 10-week observation period, 2 of 8 mice in the combined treatment group expired due to a fatal infection with aspiration pneumonia, as determined by autopsy. This is probably related to the larger volume of the oral viral dose used in this treatment group. No other side effects are observed in the remaining 34 animals.

At 14 weeks after the first NMBA dose, corresponding to 10 weeks after administration of viral recombinant FHIT, mice are euthanized; the esophagus, forestomach and other organs, including digestive tract, spleen, liver and brain are removed, and are gross anatomically evaluated by four investigators. In addition, organs are fixed in 10% formalin, and embedded in paraffin. Serial cross sections (4 µm) are prepared for histology and immunohistochemistry.
In mice treated with oral AAV-FHIT or Ad-FHIT vectors, as well as in the combined treatment group, transduction of intestinal cells is confirmed by human Fhit protein expression in murine esophagus and forestomach sections. The human Fhit protein is detected in AAV-FHIT and Ad-FHIT infected mouse esophagus and forestomach, but not in sections from the intestine of untreated mice, or in the liver, lung, or brain of any AAV-FHIT or Ad-FHIT infected mice. (Figure 2).

On gross examination, there is a marked difference between the 3 treatment groups and mice left untreated after carcinogen exposure. In the forestomach (Fst), large visible tumors, usually multiple, are seen in mice exposed to NMBA without transgene administration (Figure 3a), whereas in AAV-FHIT (Figure 3d) and Ad-FHIT treated mice (Figure 3g), as well as in the combined treatment group (Figure 3j) there is a substantial reduction both in number and size of tumors. Also, on inspection of the squamocolumnar junction (SCJ), a clear difference between the control group and the 3 treatment groups is noted.

The statistical significance of the observed differences in tumor incidence between the control and treatment groups is tested by the Fisher’s exact test (Table 1). At both predilection regions of tumor development, (the SCJ and Fst), there is a significant difference between the 3 treatment groups combined and the control group. When treatment groups are considered separately, statistically significant differences are achieved in forestomach between AAV-FHIT versus control, as well as in SCJ between Ad-FHIT versus control and AAV-FHIT versus control.

Histological examination of the esophagus and forestomach is performed on all animals to screen for signs of tumor development. In addition to the hematoxylin and eosin (H&E) staining, immunohistochemistry with the endogenous cell proliferation marker, proliferating cell nuclear antigen (PCNA) (Cels, J.E., et al., Leuk Res 10: 237-49, 1986), is performed in adjacent sections to evaluate the effect of FHIT gene therapy on carcinogen-induced proliferation associated with NMBA exposure.
Table 1. Effect of FHit gene therapy on NMBA-induced forestomach carcinogenesis in Fhit<sup>−/−</sup> mice

<table>
<thead>
<tr>
<th>Fhit&lt;sup&gt;−/−&lt;/sup&gt; animal group (no. of animals)</th>
<th>Tumor Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (12)</td>
<td>Esophagus 1/12 (8), Forestomach 11/12 (92), SCJ 8/12 (67)</td>
</tr>
<tr>
<td>Ad-FHit (8)</td>
<td>0/8 (0), 4/8 (50), 0/8 (0)</td>
</tr>
<tr>
<td>AAV-FHit (8)</td>
<td>0/8 (0), 3/8 (38), 0/8 (0)</td>
</tr>
<tr>
<td>Ad-FHit and AAV-FHit (6)</td>
<td>0/6 (0), 3/6 (50), 1/6 (17)</td>
</tr>
</tbody>
</table>

Forestomach: Ad-FHit vs Control, \( P = 0.11 \); AAV-FHit vs Control, \( P = 0.02 \); Ad-FHit + AAV-FHit vs Control, \( P = 0.08 \)
SCJ: Ad-FHit vs Control, \( P = 0.005 \); AAV-FHit vs Control, \( P = 0.005 \); Ad-FHit + AAV-FHit vs Control, \( P = 0.13 \)
Overall FHit-treatment is effective in Forestomach, \( P = 0.01 \) and in SCJ, \( P = 0.0002 \)
For each animal, total NMBA dose is 12 mg/kg (6 x 2 mg/kg, twice weekly); gene therapy is administered 10 days after the last NMBA dose. Statistical significance is analyzed by Fisher’s exact test, two-tailed

As summarized in Table 2, the pathology found in sections from control animals included papillomas (Figure 3b), focal hyperplastic lesions (FHL) and invasive carcinomas with predominance of tumors in the forestomach and at the squamocolumnar junction. In contrast, in 22 of 32 sections of AAV-FHit treated mice (Figure 3e) and in 14 of 34 sections of the combined treatment group (Ad-FHit and AAV-FHit) (Figure 3k), near normal epithelia are seen.

In some animals, carcinogen-induced changes are found after FHit transgene administration, but overall these changes are less extensive and significantly less frequent compared to the control group. An example of such a lesion, a small papilloma found in the forestomach of an Ad-FHit treated mouse, is depicted in Figure 3h. The only animal which exhibits invasive carcinoma, as well as FHL, following combined treatment (Ad-FHit and AAV-FHit) does not show murine or human Fhit expression in the tumor.

In the control group, PCNA staining reveals a substantial increase in cellular
proliferation in pathologic lesions such as FHL and papillomas (Figure 3c), whereas in the 3 treatment groups proliferation is typically confined to the basal layer (Figure 3f,i,l).

Table 2. NMBA induced phenotypes In Fhit<sup>+</sup> mice with and without FHIT gene therapy

<table>
<thead>
<tr>
<th>Fhit&lt;sup&gt;+&lt;/sup&gt; mice (no. of animals)</th>
<th>1 tumor in 12</th>
<th>1.7 tumors/mouse</th>
<th>No tumors; thickened in 1</th>
<th>FHL in all sections of all mice; papillomas in 11 mice, dys in 3, ca in 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (12)</td>
<td>12 tumors/mouse; some large</td>
<td>Multiple tumors in 8; thickened in 4</td>
<td>FHL in average of 75% of sections; near normal epithelium in average of 25% of sections</td>
<td></td>
</tr>
<tr>
<td>Ad-FHIT (8)</td>
<td>No tumors</td>
<td>1.7 tumors/mouse</td>
<td>No tumors; thickened in 2</td>
<td>FHL in average of 44% of sections; near normal epithelium in 56%</td>
</tr>
<tr>
<td>AAV-FHIT (8)</td>
<td>No tumors</td>
<td>&lt; 1.4 tumors/mouse; 1 large</td>
<td>No tumors; thickened in 1</td>
<td>FHL in 60% of sections; near normal in 40%, ca in section of 1 mouse</td>
</tr>
<tr>
<td>Ad-FHIT and AAV-FHIT (6)</td>
<td>No tumors</td>
<td>3.7 tumors/mouse; most small</td>
<td>Tumors in 1; thickened in 1</td>
<td>FHL in 60% of sections; near normal in 40%, ca in section of 1 mouse</td>
</tr>
</tbody>
</table>

At autopsy, whole esophagi and stomachs are removed and opened longitudinally. Esophageal and forestomach tumors with diameters > 0.5 mm are mapped and counted. Fst, forestomach; SCJ, squamocolumnar junction with hindstomach; ca. squamous cell carcinoma; dys, dysplasia; FHL, focal hyperplastic lesion. Tumor sizes are graded as follows. Large tumors (diameter > 2 mm), tumors (diameter 1-2 mm), and small (diameter < 1 mm). Sections, microtome sections cut across SCJ and forestomach. For each forestomach 4 to 7 sections are examined, in most cases 5 sections. Near normal epithelium denotes an epithelium that is 3-5 cells thick with mild folding at places and a thin keratin. All control sections show abundant keratin production.

Besides the carcinogen-induced pathology found in some animals of the 3 treatment groups, no additional pathology or in vivo cytotoxic effects to the FHIT transgene administration is detected, which confirms the previous in vitro observation that viral Fhit (Ad-FHITT) administration is not toxic to normal cells. (Ishii, H., et al., Cancer Res: 2000; Ji, L., et al., Cancer Res 59: 3333-9, 1999).
**Discussion**

Although two different recombinant viral vectors are used for FHit administration, macroscopic and histologic examination reveal the same biological effect with both vectors: FHit transgene results in a significant protection of the forestomach and squamocolumnar junction (SCJ) against NMBA-induced tumor development in Fhit<sup>−/−</sup> mice. Some studies with adenoviral vectors have described virus related effects such as cellular immune responses triggered by viral proteins (Yang, Y., et al., *Proc Natl Acad Sci U S A* 91: 4407-11, 1994), innate immune mechanisms (Wolff, G., et al., *J Virol* 71: 624-9, 1997), and direct cytotoxicity caused by expression of viral genes. (Muruve, D.A., et al., *Hum Gene Ther* 10: 965-76, 1999). In contrast, recombinant AAV vectors, which are devoid of all viral genes, minimize the possibility of recombination and viral gene expression. (Grimm, D., et al., *Hum Gene Ther* 9: 2745-60, 1998). Also, in vivo host immune responses to AAV thus far described are minimal. (Grimm, D., et al., *Hum Gene Ther* 9: 2745-60, 1998). The same biological effect is seen with both vector systems, thus immunological responses or direct viral effects do not contribute to the protective effect seen with both Ad and AAV vectors of the present invention.

Consistent with the two-hit model, where a somatic mutation superimposed on a germline mutation of a tumor suppressor gene leads to tumor development, the in vivo study in Fhit knock-out mice demonstrated that carcinogen-induced loss of expression of the second Fhit allele leads to tumor development. (Fong, L.Y., et al., *Proc Natl Acad Sci U S A* 97: 4742-7, 2000). The present invention reveals that recombinant viral FHit gene delivery will inhibit tumor development, thereby providing for therapeutic applications of the present invention.

Using different transgenes, adenoviral systems have been used previously in human clinical cancer gene therapy trials. In addition, a recent human phase II clinical trial reported that intra-tumoral injection of selectively-replicating adenovirus in combination with chemotherapy resulted in objective response. (Khuri, F.R., et al., *Nat Med* 6: 879-85, 2000). Since the efficacy of selectively replicating viruses can be increased by the expression of therapeutic transgenes from the virus itself (Wildner, O., et al., *Cancer Res* 59: 5233-8, 1999), FHit is a good candidate.

Also, the prominent in vivo biological response seen with AAV-FHit in the

The present invention relates the treatment of early stages of cancer, as well as to the prevention of cancer, by inhibiting tumor development. Inhibition of tumor development is achieved by FHIT gene therapy. Specifically, the local delivery of the FHIT gene will be effective in treating human premalignant lesions and cancers in which loss of Fhit is implicated. Alterations of FHIT transcripts are observed in 86% of Barrett’s metaplasia, a premalignant lesion of the esophagus, and in 93% of esophageal adenocarcinomas. (Michael, D., et al., Oncogene 15: 1653-9, 1997; Mori, M., et al., Cancer Res 60: 1177-82, 2000). This is in accordance with the high proportion of pre-malignant and malignant esophageal lesions lacking Fhit expression. (Mori, M., et al., Cancer Res 60: 1177-82, 2000). It is therefore tempting to relate the observed effect in the well-established rodent NMBA model of esophageal and gastric cancer to the human condition. In humans, lung cancer has been extensively studied for Fhit expression. (Huebner, K., et al., Advances in Oncology 15: 3-10, 1999). Most pre-neoplastic lesions, as well as more than 85% of squamous cell lung cancers, have lost Fhit expression.

(Sozzi, G., et al., Clin Cancer Res 5: 2689-92, 1999). In addition, loss of Fhit expression is more frequent in tumors of smokers. (Huebner, K., et al., Advances in Oncology 15: 3-10, 1999). As Fhit is lost very early in lung carcinogenesis, FHIT gene delivery as a therapeutic target, as well as a preventive treatment for these lesions is an object of the present invention.
CLAIMS

What is claimed is:

1. A method of *in vivo* gene therapy for treating a disorder involving a premalignant or malignant condition in a mammal, comprising:
   a) administering to said mammal a therapeutically effective amount of a viral vector comprising a *Fhit* gene, or derivative thereof;
   b) expressing a *Fhit* protein, or derivative thereof, encoded by said *Fhit* gene, or derivative thereof, in said mammal; and
   c) inhibiting a tumor development.

2. The method of Claim 1 wherein said viral vector is at least one of the group of a recombinant adenovirus or recombinant adeno-associated virus.

3. The method of Claim 1 wherein said viral vector integrates into cell genome.

4. The method of Claim 1 wherein said viral vector remains episomal.

5. The method of Claim 1 wherein said mammal is a human.

6. A method of *ex vivo* gene therapy for treating a disorder involving a premalignant or malignant condition in a mammal, comprising:
   a) transferring a *Fhit* gene to cells in tissue culture;
   b) isolating transduced cells wherein said transduced cells are cells that have taken up and express a *Fhit* protein, or derivative thereof, encoded by said *Fhit* gene, or derivative thereof;
   c) administering to said mammal a therapeutically effective amount of said transduced cells; and
   d) inhibiting a tumor development.

7. The method of Claim 6 wherein said mammal is a human.
8. The method of Claim 6 wherein said FHIT gene is integrated into cell genome.


10. A method of in vivo gene therapy for treating a predisposition to a disorder involving a premalignant or malignant condition in a mammal, comprising:
    a) administering to said mammal a therapeutically effective amount of a viral vector comprising a FHIT gene, or derivative thereof;
    b) expressing a Fhit protein, or derivative thereof, encoded by said FHIT gene, or derivative thereof, in said mammal; and
    c) protecting against induction of tumor development.

11. The method of Claim 10 wherein said viral vector is at least one of the group of a recombinant adenovirus or recombinant adeno-associated virus.

12. The method of Claim 10 wherein said viral vector integrates into cell genome.

13. The method of Claim 10 wherein said viral vector remains episomal.

14. The method of Claim 10 wherein said mammal is a human.

15. A method of ex vivo gene therapy for treating a predisposition to a disorder involving an overproliferation of cells in a mammal, comprising:
    a) transferring a FHIT gene to cells in tissue culture;
    b) isolating transduced cells wherein said transduced cells are cells that have taken up and express a Fhit protein, or derivative thereof, encoded by said FHIT gene, or derivative thereof;
    c) administering to said mammal a therapeutically effective amount of said transduced cells; and
    d) protecting against induction of tumor development.

16. The method of Claim 15 wherein said mammal is a human.
17. The method of Claim 15 wherein said \textit{FHIT} gene is integrated into cell genome.

18. The method of Claim 15 wherein said \textit{FHIT} gene remains episomal.

19. An adenovirus vector expressing a Fhit protein, or derivative thereof, encoded by said \textit{FHIT} gene, or derivative thereof.

20. An adeno-associated virus vector expressing a Fhit protein, or derivative thereof, encoded by said \textit{FHIT} gene, or derivative thereof.
Fig. 3