Gene therapy can treat obesity in mammals. An obesity regulating gene is delivered to a mammal. Preferably, the gene encodes leptin or a leptin receptor. The protein which is delivered and expressed in vivo is more effective than protein which is injected into the animal.
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TITLE OF THE INVENTION
GENE THERAPY FOR OBESITY

CROSS-REFERENCE TO RELATED APPLICATIONS
Not Applicable

STATEMENT REGARDING FEDERA LLY-SPONSORED R&D
Not Applicable

REFERENCE TO MICROFICHE APPENDIX
Not Applicable

FIELD OF THE INVENTION
This invention relates to methods of gene therapy for obesity. This invention also relates to vectors useful in this gene therapy.

BACKGROUND OF THE INVENTION
Leptin is a protein expressed by the ob gene. Leptin is secreted by adipose tissue and appears to be both a satiety factor and a regulator of metabolism (Levin et al., 1996 Proc. Natl Acad. Sci. USA 93:1726-1730). Both the mouse gene and its human homologue have recently been identified and sequenced (Zhang et al., 1994 Nature (London) 372:425-431.)

Mice which are homozygous for the ob gene (ob/ob) are obese, perhaps due to an underexpression of leptin. When ob/ob mice are given daily injections of recombinant protein, their food intake was markedly inhibited and they experienced a reduction in body weight and fat. In lean (i.e. wild-type) mice, daily injections of leptin lead to modest decreases of food intake and body weight. The results for body fat have been contradictory. (Pellemounter et al., 1995, Science 269:540-543; Halaas et al., 1995 Science 269: 543-546; and Campfield et al., 1995 Science 269:546-549.
Obesity in humans is a major disorder associated with mortality, and may result from a number of causes, and at least some may be due to an insufficient amount of leptin produced. Since leptin is a protein, and vulnerable to breakdown and inactivation by the gastrointestinal system, it cannot be delivered orally. It would be desirable to develop a therapy for leptin delivery for obese patients whose obesity is due, in part to a paucity of leptin.

Some forms of obesity do not appear to be treatable by the administration of leptin. In these cases, it is possible that the problem may be due to an insufficient amount of leptin receptors on the cell surface. Alternatively, the receptors which are present on the cell surface may contain mutations which do not allow them to bind to leptin efficiently or efficiently process the signal generated by the leptin binding. Currently no therapy exists which could augment or replace these receptors.

SUMMARY OF THE INVENTION
Not Applicable

BRIEF DESCRIPTION OF THE INVENTION
This invention is related to gene therapy for obesity. One aspect of this invention involves a method of treating obesity, lowering serum glucose levels or lowering serum insulin levels in a mammal in need of such therapy comprising delivering a gene encoding an obesity regulating gene to said mammal; and allowing sufficient time to pass for transcription and translation of the obesity regulating gene.

Some types of obesity are caused by an insufficient amount of leptin or an insufficient amount of functional leptin receptors present on the cell surface. Thus, another aspect of this invention is a method of treating obesity comprising delivering a gene encoding leptin or a leptin receptor to a mammal; and allowing sufficient time to pass for transcription and translation of the leptin or leptin receptor gene.
A specific embodiment of this invention is gene therapy for a mammal whose obesity, elevated level of serum glucose or elevated level of insulin is due, at least in part, to an insufficient amount of leptin produced. By "insufficient amount of leptin produced" it is envisioned that the animal may produce functional leptin, but at lower levels than required, a complete inability to produce leptin, or production of a mutated form of leptin which either functions less efficiently than native leptin or does not function at all. Thus, this invention is directed to a method of treating obesity, an elevated level of serum glucose or an elevated insulin, which is, at least in part, due to an insufficient amount of leptin produced by a mammal comprising: delivering a gene encoding leptin to the mammal and allowing sufficient time to pass for transcription and translation of the leptin gene.

Obesity may occur in an animal which is producing normal quantities of leptin, but whose leptin receptors are either not able to bind and process the leptin properly, or have not been produced in sufficient quantity. These situations may be remedied by gene therapy using a leptin receptor. Thus another aspect of this invention is a treatment for obesity, excess plasma insulin levels or excess plasma glucose levels, any of which are a result, at least in part, of an insufficient amount of functional leptin receptor production by a mammal. Thus, one aspect of this invention is a method of increasing the amount of leptin receptors in a mammal comprising: delivering a gene encoding a leptin receptor to the mammal, and allowing sufficient time to pass for transcription and translation of the leptin receptor gene.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a photograph of two ob/ob mice. The mouse on the right is from a control group. The mouse on the left received gene therapy in accordance with this invention.

Figure 2 is a graph showing the body weight changes of mice treated with recombinant human leptin protein. Injections of human recombinant leptin were given daily IP, at 1 μg/gm body weight. Arrows indicate bleeding points.
Figure 3 is a graph showing body weight changes of mice treated with adenovirus carrying a reporter gene (β-galactosidase), used as a control.

Figure 4 is a graph showing body weight changes of mice treated with adenovirus carrying the human leptin gene.

Figure 5 is a graph showing the percent of body weight changes for all groups of mice.

Figure 6A (left graph) is a graph showing the amount of human leptin found in the plasma of mice treated with adenovirus containing the leptin gene. Figure 6B (right graph) shows results for mice treated with five daily injections of recombinant human leptin.

Figure 7A (left graph) shows the amount of insulin and leptin in plasma of mice treated with adenovirus containing the leptin gene. Figure 7B (right graph) shows the amount of insulin and leptin in plasma of mice treated with recombinant human leptin injections.

Figure 8 shows glucose levels of the mice treated with either recombinant leptin, reporter gene or adenovirus containing the leptin gene.

As used throughout the specification and claims, the following definitions apply:

"Native": a gene or protein is native if it naturally occurs in a given organism.

"Leptin gene": a gene from any mammal which encodes a native leptin, or a derivative thereof. A "derivative" is a modified leptin molecule which retains at least 80% of the biological activity of native leptin.

"Leptin receptor": a gene from any mammal which encodes a native leptin receptor, or a derivative thereof. A "derivative" is a modified receptor molecule which binds native leptin at least 80% as efficiently as a native receptor molecule.

"Obesity regulating gene": a gene whose gene product is involved in the regulation of obesity in a mammal, including genes encoding leptin, leptin receptors, neuropeptide Y, and the like.
In the past, recombinant leptin has been administered to animals who exhibiting an obese phenotype, and a daily injection has been shown to decrease body weight. There are numerous disadvantages to this method of treating obesity, however. First, this method is helpful only for those animals whose obesity is caused, at least in part by an insufficient amount of leptin produced. Not all obesity is due to this defect. Further, injections are not a particularly convenient method of treatment, particularly for long-term treatments. In addition, the half-life of leptin is short, so the duration of the treatment was found to be only about 24 hours, after which the animals were observed to regain weight.

This invention solves the problems associated with a daily administration of recombinant protein by providing a vector which can express leptin or a leptin receptor in vivo. It has been surprisingly found that leptin which is expressed in vivo is more advantageous than administration of recombinant leptin; its effects last longer, and most surprisingly, is up to 20 fold more potent than recombinant leptin administered by injection.

Expression of a leptin receptor or neuropeptide Y in vivo allows for treatment of heretofore untreatable types of obesity.

**Genes**

The sequences of leptin and leptin genes from various species are known (Zhang et al., 1994 Nature 372:425; Ogawa et al., 1995 J. Clin. Invest. 96:1647-1652; Murakami et al., 1995 Biochem. Biophys. Res. Commun. 209:944; and Considine et al., 1995 J. Clin. Invest. 95:2986; each of which is hereby incorporated by reference). If desired, genes encoding leptin derivatives may also be used. Since the amino acid and nucleotide sequence of leptin is known, it is well within the skill of one of the ordinary artisan to construct a nucleotide sequence which encodes a desired mutant form of leptin. These can be used to study structure and function relationships involved in leptin binding and signaling in the transgenic animal model.
The amino acid sequences of leptin receptors and the nucleic acid sequences of genes encoding leptin receptors are also known; see, for example Toriaglia et al., 1995, Cell 83:1-20 which is hereby incorporated by reference. The leptin receptors can exist in various isoforms, due to alternate splicing. The biological consequences of the presence of many of these isoforms is not clearly understood. However, a mutation that results in premature termination of the long form of the mouse leptin receptor is apparently responsible for the obese phenotype of the db/db mouse (Lee et al., 1996, Nature 379:632-635; Chua et al., 1996, Science 271:994-996; and Chen et al., 1996, Cell 84:491-495). In further aspects of this invention, a derivative leptin receptor is introduced into a mammal, and the resulting mammal can be used to study structure and functional relationships between leptin binding and the leptin receptor.

The gene which encodes the leptin or leptin receptor should also contain at least one element which allows for expression of the gene when introduced into the host cell environment. These sequences include, but are not limited to promoters, response elements, and enhancer elements. In a preferred aspect of this invention, promoters are chosen which are regulatable; i.e. are inducible rather than constitutive. Particular examples of such promoters include: Sr-alpha, CMV, regulatable tet, P-450, albumin and the like.

**Vector**

The heterologous leptin or leptin receptor gene may be delivered to the organism using a vector or other delivery vehicle. DNA delivery vehicles can include viral vectors such as adenoviruses, adeno-associated viruses, and retroviral vectors. See, for example: Chu et al. 1994 Gene Ther 1: 292-299; Couture et al. 1994 Hum Gene Ther 5:667-677; and Eiverhand et al. 1995 Gene Ther 2: 336-343. Non-viral vectors which are also suitable include DNA-lipid complexes, for example liposome-mediated or ligand/poly-L-Lysine conjugates, such as asialoglyco-protein-mediated delivery systems. See, for example:
Felgner et al. 1994 J. Biol. Chem., 269: 2550-2561; Derossi et al. 1995, 
Restor. Neurol. Neuros. 8: 7-10; and Abcallah et al. 1995 Biol. Cell 85: 
1-7.

If a vector is chosen as the delivery vehicle for the obesity 
regulating gene, it may be any vector which allows expression of the 
gene in the host cells. It is preferable if the vector also is one which is 
capable of integrating into the host genome, so that the gene can be 
expressed permanently, but this is not required. In cases where the 
vector does not integrate into the host genome, the expression of the 
gene may be transient rather than permanent.

One vector which is suitable for transient expression of the 
ob gene is an adenovirus which has a deletion in the E1 gene. Such 
vectors are known, as taught in the aforementioned WO 95/00655 and 
Mitani et al., 1995 publications. These viruses preferentially infect 
hepatocytes, where they persist for approximately 3-4 weeks after the 
initial infection. While in the hepatocytes, these viruses can express the 
heterologous gene.

The vector is administered to the host, generally by IV 
injection. Suitable titers will depend on a number of factors, such as the 
particular vector chosen, the host, strength of promoter used and the 
severity of the disease being treated. For mice, an adenovirus vector is 
preferably administered as an injection at a dose range of from about 
5.0 x 10^6 to about 10 x 10^6 plaque forming units (PFU) per gram body 
weight. Preferred dosages range from at least about 6-9 x 10^6 PFU/gm 
body weight, and more preferred is from at least about 6.7-8.6 x 10^6 
PFU/gm body weight (equivalent to approximately at least 1-5 x 10^8 
PFU for mice).

Thus this invention specifically is directed to a method of 
treating obesity, elevated serum glucose levels or elevated insulin levels 
which is, at least in part, due to an insufficient amount of leptin or leptin 
receptors by a mammal comprising:

a) transfecting the mammal with a viral vector 
comprising a gene encoding leptin or leptin 
receptors; wherein said vector further comprises at
least one element regulating its expression in a mammalian tissue; and
b) allowing sufficient time to pass for transcription and translation of the leptin gene.

5 Hosts

Animals which transiently express the ob or ob-receptor gene products are valuable research tools. For example, they can be used to monitor the effects of decreasing amounts of leptin, or the effect of various exogenously supplied substances (such as hormones or putative leptin receptor agonists and antagonists) in an environment of decreasing leptin availability.

Aside from making animal models useful in studying various aspects of obesity, this invention is specifically directed to gene therapy for humans.

In accordance with this invention, mice which are obese (ob/ob) have been injected with an adenovirus containing the human leptin gene, although the leptin gene from any desired species may be used, and in preferred embodiments, the gene which is from the same species as the host is used. These were compared with ob/ob mice injected with an adenovirus containing only a marker gene (β-galactosidase), those receiving injections of recombinant leptin, and to untreated controls. Further controls used in some of the experiments are db/db mice (obese, but unresponsive to leptin injections).

Body-weight: Figure 2 illustrates the body weight changes for mice receiving 1 μg/gm body weight human recombinant leptin protein injections daily, compared to untreated controls. Animals receiving leptin were injected for five consecutive days, shown by the darkened symbols on the graph. All the animals receiving the leptin lost weight within 24 hours post-injection. All animals gain weight within 48 hours after the last IP injection. Figure 3 illustrates the weight measured for mice receiving various titers of an adenovirus carrying
the reporter gene. All animals continued to gain weight post injection. Figure 4 shows the results for mice receiving an adenovirus carrying the leptin gene. As can be seen from the graph, all animals lost weight within 24 hours post injection. They continued to loose weight for 1-2 weeks post treatment. The injections were effective over a relatively large titer range, and a dose-effect was noted.

Along with absolute changes in weight, the percentage body weight change was calculated for all groups. In the animals treated with recombinant leptin injections, weight loss plateaued at day three, and from day 1-5 post treatment, a 4.7% loss in body weight was noted. In those mice treated with the vector carrying the leptin gene, weight loss persisted over a 10-12 day period, and resulted in an 18.61% loss in body weight. Furthermore, over days 1-5 post treatment, a 9.17% loss in body weight was observed compared to only 4.7% loss in the recombinant leptin treated mice. This is illustrated in Figure 5.

**Leptin:** The amount of human leptin in plasma was measured in the animals which received injections of human recombinant leptin and those which received the vector carrying the leptin gene. Those receiving the recombinant protein were noted to have leptin levels which were approximately 20-fold higher than the amount of leptin found in control (lean, wild type) animals; peak amounts of 399.8 ± 40.91 ng/ml. Those receiving the leptin gene had levels of leptin in their plasma which was within the normal range found in a wild-type mouse (17.52 ± 4.66 ng/ml). In both groups of animals, weight gain was synchronized with the fall of human leptin detected in the plasma. This is illustrated in Figure 6.

**Insulin:** The amount of insulin in the plasma was measured in both the animals receiving recombinant protein and those which received the gene therapy. This is illustrated in Figure 7. In both groups, insulin levels were observed to drop to those found in lean (wild-type) levels and was inversely correlated to leptin levels. In the mice receiving gene therapy, the low insulin levels were sustained for at
least one week whereas in the recombinant leptin-treated mice, insulin levels increased to pre-treatment levels within 24 hours post injection.

**Glucose:** The levels of glucose in plasma was also measured in mice receiving recombinant leptin and those receiving the gene therapy treatment. In both groups, the glucose levels dropped within 6-9 days post treatment. The recombinant protein-treated mice did not achieve levels comparable to those found in lean, wild-type mice, and only sustained the lower level for less than one week. On the other hand, the mice which received the gene therapy had reductions in glucose levels to that of wild type lean mice, and they sustained this reduced level for at least two weeks. This is illustrated in Figure 8.

The following non-limiting Examples are presented to better illustrate the invention.

**EXAMPLE 1**

**Cloning and expression of leptin**

Two PCR cDNA amplification fragments were obtained from Jefferson University (generated by cloning both variants from a Clontech phage human hypothalamic library): one coding for the human leptin and one for the human leptin variant with glutamine, (Zhang et al., 1994, Nature 372:425; Considine et al., 1995 J. Clin. Invest. 95:2986). Both PCR fragments were amplified for cloning purposes.

Two primers were designed and ordered from GIBCO BRL Custom Primers: Forward primer: ATG CAT TGG GGA ACC CTG TG
Reverse primer: TCA GCA CCC AGG GCT GAG GT

The primers were used to re-amplify the cDNA as follows: 2 μl each primer (0.3 μg/μl stock), 2 μl dNTP (10 μM, Pharmacia), 10 μl 10 X PCR Buffer (Buffer 2 from Expand Long Template PCR System Kit, Boehringer Mannheim), 2 μl Taq polymerase (Perkin Elmer), 3 μl template DNA and 18 μl water. PCR cycling conditions were as follows: Mixture was incubated at 94°C initially (without the addition of the Taq enzyme) for 1-2 minute, Taq was then added to each tube and the cycling program was initiated, 20 cycles of 94°C for 30
seconds, 45°C for 45 seconds and 72°C for 1 minute. At the end of the 20 rounds of amplification the samples were incubated at 72°C for 7 minutes. The expected fragment size in each case (human leptin - hOb, and human leptin with glutamine-hObGLN) was 501 and 504, respectively. The PCR amplified fragments were cloned into pCR-Script SK(+) plasmid (Stratagene) and several selected bacterial colonies were grown, plasmids extracted and sequenced to verify correct sequence of both cloned.

Inserts were then used for generating recombinant adenoviral shuttle vectors. The adenoviral vectors used in this study are essentially the same as those described in Morsey et al 1993 J. Clin. Invest. 92: 1580-86, which is hereby incorporated by reference, except for the leptin gene insert. The pdelE1sp1CMV-BGHpA adenoviral shuttle vector, obtained from Baylor College of Medicine was used for the cloning of the two inserts (hOb and hObGLN). Similarly mOb cDNA (Zhang et al., 1994 Nature 372:425) was inserted into pdelE1sp1CMV-BGHpA. All three shuttles (pdelE1sp1CMV-mOb-BGHpA, pdelE1sp1CMV-hOb-BGHpA and pdelE1sp1CMV-hObGLN-BGHpA) were tested for leptin expression by western blot analysis.

**EXAMPLE 2**

The three shuttle vectors from the previous example are used in rescue replication of the deficient E1 deleted adenoviral vectors. 293 cells, commercially available from Microbix, passage 27-30 were set up one day ahead of transfection in 60mm dishes, and were about 70-80% confluent at the time of use.

Plates were made containing one of the shuttle plasmids and pJM17; pFG140 (purchased from Microbix Biosystems Inc.) was used as a positive control for the efficiency of transfection. Plaques were identified, and plugged out of the agarose overlay using a sterile glass Pasteur pipette. Each plugged plaque was resuspended in 100-500 µl of PBS (with calcium and magnesium) in 10% glycerol, frozen at -80°C and thawed (1-3 times). The thawed plaque was then used to infect a 90% confluent 6 cm plate of 293 cells to expand the isolated virus. 5-8
days post infection, cytopathic effects (cpe) were apparent on the cells (cells rounded up and started to detach and float in media). Cells were collected by scraping and tested for leptin expression by western analysis and for DNA restriction pattern by Hind III digestion of extracted DNA and ethidium bromide stained gel analysis. One of the positive plaques identified based on leptin secretion and correct DNA restriction pattern was selected and used for a second plaque purification followed by a similar procedure of expansion and analysis. After the second plaque purification, the virus was propagated on a large scale. Cesium banding and titration was used to purify and quantitate.

The resulting titered viral stocks (Ad-HCMV-mOb-BGH<sup>PA</sup>, Ad-HCMV-hOb-BGH<sup>PA</sup> and Ad-HCMV-hOb GLN-BGH<sup>PA</sup>) were stored at -80°C until use.

**EXAMPLE 3**

**Transgenic mice**

**Baseline determinations.** Three groups of mice were used: *ob/ob, db/db*, and lean (wild type, controls). All groups of mice were fed milled rodent chow (5008) starting from day of arrival or day after arrival. Food consumption was also measured. After approximately 4 days on milled chow, the mice were weighed and bled for determination of plasma levels of glucose and insulin. Injections were started 8 days after the initiation of base line measurements but before injections, mice were weighed and blood samples were obtained from all study mice for determination of plasma glucose and insulin. Leptin level in plasma were also measured.

Mice were housed 5 per cage and fed milled Purina Chow 5008 in feed cups with lids. 24 hour food consumption was measured at the same time each day. Only after food consumption was equilibrated to a fairly constant level, usually 20-25 grams chow/5 mice-day, was virus injected.
On the day of injection but before injection, food consumption, body weight, and a baseline blood sample were taken in the morning from a snipped end of tail. Blood was collected into heparinized capillary tube (total volume approximately 70-100µl). Hematocrit was measured, and plasma was collected.

Mice were injected as follows:

A. **ob/ob mouse groups: iv injections, 5 mice/group**
   - Group 1 received $2.75 \times 10^8$ / gm wt of AdHCMV-hob-BGH$_{P4}$ (in 500µl dialysis buffer) in the tail vein.
   - Group 2 received $2.75 \times 10^8$ / gm wt of AdHCMV-βgal reporter (in 500µl dialysis buffer) in tail vein.
   - Group 3 received 500µl dialysis buffer in tail vein.
   - Group 4 received 1 mg/kg wt active leptin daily IP injections for 5 days.

B. **db/db mouse groups: iv injections, 5 mice/group**
   - Group 1 received $2.75 \times 10^8$ / gm wt of AdHCMV-hob-BGH$_{P4}$ (in 500µl dialysis buffer) in the tail vein.
   - Group 2 received $2.75 \times 10^8$ / gm wt of AdHCMV-βgal reporter (in 500µl dialysis buffer) in tail vein.
   - Group 3 received 1 mg/kg wt active leptin daily IP injections for 5 days.

C. **Lean control mouse group: one cage of 5 mice as measure of lean parameters**
   - No injections.

**EXAMPLE 4**

**Leptin receptor**

Adenovirus vectors are made similarly to those described in Examples 2-3, except that the leptin receptor gene replaces the leptin gene. Mice which are db/db are used in place of the ob/ob mice. Results for the db/db mice are similar to those observed with the ob/ob
mice reported herein. After injection, glucose levels fall, insulin levels fall and the mice loose weight. No effect is observed in control mice and in ob/ob mice injected with vector carrying a leptin receptor gene.

5 DETAILED DESCRIPTION OF THE INVENTION
Not Applicable
WHAT IS ClaimED:

1. A method of treating obesity, lowering serum glucose levels or lowering serum insulin levels in a mammal in need of such therapy comprising delivering an obesity regulating gene to the mammal; wherein transcription and translation of the gene occurs in vivo.

2. A method of treating obesity, lowering serum glucose levels or lowering serum insulin levels in a mammal in need of such therapy comprising delivering a gene encoding a leptin to the mammal; wherein transcription and translation of the gene encoding leptin occurs in vivo.

3. A method according to Claim 2 wherein the heterologous gene encoding a leptin is delivered in a vector.

4. A method according to Claim 3 wherein the vector is an adenovirus.

5. A method according to Claim 4 wherein the adenovirus is expressed in liver.

6. A method according to Claim 1 wherein the mammal is a human.

7. A method of treating obesity which is, at least in part, due to an insufficient amount of leptin receptors produced by a mammal comprising:

    a) transfecting the mammal with a viral vector comprising a gene encoding a leptin receptor; wherein said vector further comprises at least one element regulating its expression in a mammalian tissue; and
b) allowing sufficient time to pass for transcription and translation of the leptin receptor gene.

8. A method according to Claim 7 wherein the vector is a viral vector.

9. A method according to Claim 8 wherein the vector is an adenovirus.

10. A method according to Claim 9 wherein the adenovirus is expressed in liver.

11. A method according to Claim 10 wherein the mammal is a human.

12. A vector comprising a gene encoding a leptin receptor; wherein said vector further comprises at least one element regulating its expression in a mammalian tissue.

13. A method according to Claim 7 wherein the vector is an adenovirus.
FIG. 1

Adeno-mediated human leptin delivery and expression in ob/ob mice
Phenotypic Correction 10 Days Post Treatment

ob/ob treated with a single IV dose (2.75x10^6 pfu) of Ad-HCMV-hOb

Untreated ob/ob or treated with control virus Ad-HCMV-βgal
DAILY IP INJECTIONS

BODY WEIGHT IN GRAMS

DAYS

ob/ob - UNTREATED

ob/ob - RECOMBINANT HUMAN LEPtin PROTEIN

1ug/gm

BLEEDING POINTS

FIG. 2

SUBSTITUTE SHEET (RULE 26)
FIG. 3

PFU/gm BODY WEIGHT

- 6.7x10^6/gm
- 7x10^6/gm
- 8.4x10^6/gm
- 8.6x10^6/gm
- 8x10^6/gm

IV INJECTION

BLEEDING POINTS

DAYS

BODY WEIGHT IN GRAMS

30

35

40

45

50
FIG. 4

SUBSTITUTE SHEET (RULE 26)
FIG. 5

SUBSTITUTE SHEET (RULE 26)
FIG. 8

IP INJECTION OF RECOMBINANT HUMAN LEPTIN (1 μg/gm BODY WT)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
   IPC(6) : A61K 48/00; C12N 15/12, 15/86
   US CL : 435/320.1; 424/93.2; 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. : 435/320.1; 424/93.2; 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)
   APS; DIALOG DATABASES: BIOSIS, MEDLINE, CA SEARCH, WORLD PATENT INDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<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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<tbody>
<tr>
<td>X</td>
<td>WO 96/05309 A2 (THE ROCKEFELLER UNIVERSITY) 22 February 1996, see entire document.</td>
<td>1-6</td>
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</tbody>
</table>

[X] Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search: 02 SEPTEMBER 1997

Date of mailing of the international search report: 31 OCT 1997

Name and mailing address of the ISA/US
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<td>X,P</td>
<td>WO 97/19952 (MILLENNIUM PHARMACEUTICALS, INC.) 05 June 1997, see entire document, especially pages 43 and 45.</td>
<td>12, 1,7-11,13</td>
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