A61K 3/00 (2006.01) A61K 3/22 (2006.01)

(21) International Application Number:
PCT/IB20 14/06951

(22) International Filing Date:
23 April 2014 (23.04.2014)

(25) Filing Language:
English

(26) Publication Language:
English

(30) Priority Data:
106908 23 April 2013 (23.04.2013) PT

(71) Applicant: UNIVERSIDADE DE COIMBRA [PT/PT]; Reitoria - Paço das Escolas, P-3004-53 1 Coimbra (PT).

(72) Inventors: GONCALVES CAVADAS, Claudia Margarida; Rua Antero de Quental, 117, P-3000-032 Coimbra (PT). MORGADO PEREIRA DE ALMEIDA, Luís Fernando; Rua Antero de Quental, 117, P-3000-032 Coimbra (PT). FERREIRA DE OLIVEIRA AVELEIRA, Celia Alexandra; Estrada da Beira, Lote 5, 5° Frente, P-3030-173 Coimbra (PT). RODRIGUES NOBREGA, Clevio David; Praceta Alberto Sa de Oliveira, N.2° 1-E, P-3030-035 Coimbra (PT). BOTELOH DA ROCHA, Mariana; Estrada Principal No. 41 Horto, P-3800-719 Eixo (PT). MATIAS CARMO SILVA, Sara; Rua Salvador Tavares Machado, 20 Frente, P-3720-280 Oliveira De Azeméis (PT).

(74) Agent: VIEIRA PEREIRA FERREIRA, Maria Silvina; CLARKE, MODET & CO., Rua Castilho, 50-9°, P-1269-163 Lisboa (PT).


Published:
— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: NEUROPEPTIDE Y OVEREXPRESSION FOR USE IN THE TREATMENT OF PREMATURE AGING

(57) Abstract: The present application describes the therapeutic effect of hypothalamic neuropeptide Y overexpression against age related pathologies progression and progeroid syndromes, namely the Hutchinson-Gilford progeria syndrome. The application also describes the use of the hypothalamic neuropeptide Y overexpression in the treatment of genetic or acquired lipodystrophies, such as selective loss of body fat, anorexia and cachexia, or in the treatment of alopecia, early hair loss, subcutaneous fat loss, bone dysfunc-
tion and recognition memory.

Figure 1

(51) International Patent Classification:
C07K 14/575 (2006.01) A61K 48/00 (2006.01)

(52) Convention family:
A44-NPY-injected (NPN) Female — Cypionate 17° Male
A44-NPY-injected (NPN) Female — Cypionate 17° Female
A44-NPY-injected (NPN) Female — T23y88525° Female

(53) Designated State family:
DESCRIPTION
"NEUROPEPTIDE Y OVEREXPRESSION FOR USE IN THE TREATMENT OF
PREMATURE AGING"

Technical Field
The present application relates to the neurosciences filed, namely gerontology.

Background
Normal aging and premature aging, that characterizes progeria syndromes, share similar features. Hutchinson-Gilford progeria syndrome (HGPS) is a rare condition with features of premature and accelerated aging in children that show short stature, low body weight, early hair loss, dermal and bone abnormalities, lipodystrophy (lack of subcutaneous fat), bone and cardiovascular dysfunction, and atherosclerosis, all leading to a shortened lifespan (Hennekam, 2006). HGPS is primarily caused by a de novo silent mutation within exon 11 of the LMNA gene encoding lamin A (c.1824C>T;p.Gly608Gly), resulting in the synthesis of a truncated form of prelamin A, called LAD50 or progerin, which lacks a 50-residue-long fragment containing the cleavage site for ZMPSTE24 (Desandre-Giovannoli et al 2003).

The involvement of an inflammatory response in HGPS has been suggested. In fact, NF-κB activation during aging has been reported in human and mouse tissues as well as in cells from HGPS patients (Tilstra et al 2012). More recently, it was also showed that NF-κB is constitutively hyperactive in Zmpste24−/− mice tissues (Osorio et al., 2012). Consistent with the observed activation of NF-κB signaling in this progeroid mice model, several cytokines
and adhesion molecules, such as Cxcr2, IL-6, CXCL1, and TNF-alpha are strongly up-regulated in cells and tissues from these mice, likely contributing to the initiation and maintenance of an inflammatory response (Osorio et al 2012).

The hypothalamus is a key brain region crucial for body weight regulation through energy balance regulation (food intake and energy expenditure). The arcuate nucleus (ARC) neurons of the hypothalamus have a key role in sensing and integrating peripheral signals (i.e., leptin, ghrelin or insulin) (Schwartz et al 2000). The neuropeptide Y (NPY) is one of the most abundant peptides in the mammalian brain (Silva et al. 2005), especially in the ARC of hypothalamus (Allen et al. 1983), where it is involved in the central regulation of energy balance and stimulates food intake resulting in a significant increase in body weight and adiposity (Sousa-Ferreira et al 2011). ARC neurons include two distinct populations acting together to regulate feeding behavior: the orexigenic NPY/AGRP (Neuropeptide Y/Agouti-Related Protein) neurons and the anorexigenic POMC/CART (Pro-OpiMelano cortin/Cocaine - and Amphe famine - Responsive-Transcript) neurons. Several conditions (dys)regulate the function of these two neurons population leading to energy balance (dys)regulation such as: peripheral hormones (leptin, insulin, adiponectin), energy status, obesity, caloric restriction, autophagy, and inflammation. A decrease in autophagic processes in hypothalamic neurons has been shown to contribute to the metabolic and energy balance dysregulation (Coupe et al, 2012; Quan et al, 2012). The peripheral hormones leptin and insulin, through their receptors, activate anorexigenic neurons (POMC/CART) and inactivate orexigenic neurons.
(NPY/AgRP), resulting on a decrease of food-intake. Also the inflammatory cytokine IL-1beta promotes negative energy balance (Scarlett et al 2008). Moreover, ARC may act as an inflammatory amplifier within the CNS, suggesting that neurons in the ARC are likely subject to much higher concentrations of pro-inflammatory cytokines than found in the circulation (Laye et al 1994). Moreover, hypothalamic inflammation (cytokine increase, microglia activation and astrogliosis) induced by acute or chronic systemic inflammation (ex: infection, fever) promotes negative energy balance (decrease in food intake) (Thaler et al 2010).

Very recently, Zhang et al. (2013) showed that the hypothalamus is also responsible for systemic aging and thus lifespan control. Notably the authors showed that the hypothalamus is important for the development of whole-body aging in mice, and hypothalamic microglia acts via IKK-b and NF-kB to contribute to the role of the hypothalamus in aging development. Thus, these new findings provide a proof of principle to the hypothesis that aging is a life event that is programmed by the hypothalamus via immune-neuroendocrine integration.

Summary

The present application describes the use of a hypothalamic neuropeptide Y overexpression in the treatment of age related pathologies progression.

A preferred embodiment of the present invention describes the use of a hypothalamic neuropeptide Y overexpression in therapies against progeroid syndromes, namely the Hutchinson-Gilford progeria syndrome.
In another embodiment of the present invention, the age related pathologies progression are Alzheimer's disease, Parkinson's disease or Machado Joseph disease.

A preferred embodiment of the present invention describes the use of a hypothalamic neuropeptide Y overexpression in the treatment of genetic or acquired lipodystrophies, such as selective loss of body fat, anorexia and cachexia.

In another embodiment of the present invention, the referred anorexia and cachexia are associated with other chronic conditions, for example cancer, rheumatoid arthritis, HIV infection, and chronic lung, heart, or kidney disease.

A preferred embodiment of the present invention describes the use of a hypothalamic neuropeptide Y overexpression in the treatment of alopecia, early hair loss, subcutaneous fat loss, bone dysfunction and recognition memory.

**General description**

The present application describes that a sustained increase in the endogenous levels of NPY in the hypothalamus, by using techniques of gene delivery using viral vectors, can block or reverse premature aging in human progeroid syndromes or age related pathologies progression, namely when associated in HGPS.

The chronic systemic inflammation that occurs in these syndromes induce hypothalamic inflammation or dysfunction that are responsible for the changes in various physiological functions regulated by the hypothalamus presented, such as, energy imbalance, low body weight and adiposity, and a premature and accelerated aging.
The present application describes the effect the hypothalamic neuropeptide Y (NPY) has in the aging process of Face-1/Zmpste24 deficient mice, which exhibit multiple defects that phenocopy human accelerated aging processes such as Hutchinson-Gilford progeria syndrome.

At 2 months of age, Face-1/Zmpste24^-/- mice are smaller and have a lower body weight (15.4 ± 0.3 g) compared to age-matched wild type littermates (22.8 ± 0.4 g). Since NPY is a potent orexigenic neuropeptide, to avoid major weight changes, NPY-treated Face-1/Zmpste24^-/- mice were pair-fed (given the same amount of food as non-treated-Face-1/Zmpste24^-/- mice ate, daily).

One month after NPY treatment, non-treated-Face-1/Zmpste24^-/- mice (3 month old) gained less weight (18.3 ± 0.8 g) than the NPY-treated Face-1/Zmpste24^-/- (27.7 ± 0.4 g), which reached a similar body weight to wild-typeagematched mice (26.4 ± 0.6 g). Three months after NPY treatment, non-treated-Face-1/Zmpste24^-/- mice (5 month old) still exhibited a lower body weight (17.7 ± 1.1 g) than NPY-treated Face-1/Zmpste24^-/- (27.2 ± 1.7 g).

In addition to body weight improvement, NPY treatment increased subcutaneous fat and decreased the degree of kyphosis and alopecia of Face-1/Zmpste24^-/- mice. Although NPY treatment had no effect on the locomotor activity of these mice, NPY-treated Face-1/Zmpste24^-/- mice showed a better performance in memory tasks than non-treated Face-1/Zmpste24^-/- mice.

These results demonstrate that increased hypothalamic NPY levels induce a beneficial effect, delaying characteristic features of the progeroid phenotype of Face-1/Zmpste24^-/- mice, such as body weight loss, lipodystrophy, alopecia and
memory impairment. Modulation of NPY levels act as a protective mechanism against the aging process progression and, therefore, provide a putative therapeutic strategy for the treatment of human progeroid syndromes.

**Brief description of drawings**

Without intent to limit the disclosure herein, this application presents attached drawings of illustrated embodiments for an easier understanding.

**Figure 1.** Hypothalamic NPY overexpression increases Face-1/Zmpste24^-/^- body weight. (A) Body weight of 2 and 3 months old Face-1/Zmpste24^-/^- mice and age-matched wild type littermates. (B) Body weight in male saline injected and AAV-NPY-injected Face-1/Zmpste24^-/^- mice and female saline-injected and AAV-NPY-injected Face-1/Zmpste24^-/^- mice. *p<0.05; **p<0.01, significantly different as determined by two-way ANOVA followed by Bonferroni post-test.

**Figure 2.** NPY increases adipose tissue in Face-1/Zmpste24^-/^- mice. Representative images of saline-injected and AAV-NPY-injected mice after 120 days of AAV injection. AAV-NPY-injected Face-1/Zmpste24^-/^- mice show an increase in both subcutaneous and visceral fat and gonadal fat depots.

**Figure 3.** NPY reduces fur loss in Face-1/Zmpste24^-/^- mice. (A) Representative photographs of saline-injected and AAV-NPY-injected mice after 30 and 120 days of AAV injection. (B) Fur loss was assessed twice a week and quantified by a semi-quantitative ranking score system on a scale of 0 to 3: 0 for absent fur loss, 1 for mild fur loss, 2 for moderate fur loss, and 3 for marked fur loss. The results were summed up for each animal group after 30, 60, 90 and
120 days upon AAV-NPY injection and the frequency of each ranking score was calculated and plotted on a bar chart. ***p<0.001, significantly different, as determined by chi-square test of independence.

**Figure 4.** Effect of NPY treatment in the locomotor activity and memory performance of *Face-1/Zmpste24*/*- mice. (A) Locomotor horizontal activity of mice was monitored for 30 min in the open field box. Data are expressed as mean ± SEM of total distance travelled (centimeters). (B) Spatial recognition memory was tested using the Y maze test. The percentage of time spent in the novel arm during the 8 min of the second trial was determined. Data are expressed as mean ± SEM. *p<0.05; **p<0.01 (Two-way ANOVA/Bonf erroni post-test).

**Mode(s) for carrying out embodiments**

Referring to the drawings, herein are described optional embodiments in more detail, which however are not intended to limit the scope of the present application.

Normal aging and premature aging that occurs in progeria syndromes have similar features. The Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare condition or genetic disease that manifests itself at a very early age, characterized by premature and accelerated aging characterized by short stature, low body weight, early hair loss, lack of subcutaneous fat, decreased joint mobility, osteolysis, atherosclerosis, and premature death. Early death is usually caused by cardiac complications. No robust strategy it is known to delay aging and to treat progeria syndromes progression. HGPS patients die at an
average age of 13 years and, so far, no treatment is available.
No therapeutic strategy is known to delay human aging and
to treat progeria syndromes progression, cachexia or anorexia.

The technical approach used to induce a sustained increase
of endogenous neuropeptide Y in the brain region
hypothalamus, by using gene delivery approach (viral
vectors), in aged animals delays and ameliorates age-
related deteriorations, is a new strategy to rescue and
ameliorates the age-related deteriorations (such as
alopecia, aging-anorexia, subcutaneous fat loss, bone
dysfunction, recognition memory). This methodology is a
new therapeutical approach applied to ameliorates age-
related deteriorations observed in progeria syndromes, in
normal ageing, in aged related pathologies, such as
Alzheimer's disease, Parkinson's disease, and Machado
Joseph disease, in genetic or acquired lipodystrophies,
such as disorders characterized by selective loss of body
fat, anorexia and cachexia associated with other chronic
conditions, for example cancer, rheumatoid arthritis, HIV
infection, and chronic lung, heart, or kidney disease.

Therefore, in the present application the beneficial effect
of a sustained increase of hypothalamic NPY in the aging
process was evaluated by overexpressing NPY in the arcuate
nucleus of hypothalamus, using Adeno-Associated Viral (AAV)
gene transfer technology, in Zmpst24- null mice, a mouse
model of premature-aging. Zmpst24 (also called FACE-1) is
a metalloproteinase involved in the maturation of lamin A,
an essential component of the nuclear envelope. Zmpst24-
deficient mice exhibit multiple defects that phenocopy
human accelerated aging processes such as Hutchinson-Gilford progeria syndrome. Zmpste24-deficient (Zmpste24-) mice exhibit retarded growth, alopecia, micrognathia, dental abnormalities, lipodystrophy, muscular dystrophy, cardiomyopathy and osteoporosis. Most Zmpste24- mice died prematurely, having an average lifespan of 5-6 months. Anorexia and cachexia are common complications of aging and other chronic conditions including cancer, rheumatoid arthritis, HIV infection, and chronic lung, heart, or kidney disease. An increase of endogenous hypothalamic NPY, that stimulates food intake and adiposity, is a putative relevant strategy to be applied in anorexia and cachexia.

The Hutchinson-Gilford progeria syndrome (HGPS) is characterized by clinical features mimicking physiological aging at an early age, provide insights into the mechanisms of natural aging. The following experiments show that the sustained increase of hypothalamic NPY, by using gene delivery approach, in mouse model (Zmpste24-deficient mice) of human accelerated aging ameliorates age-related deteriorations and has applicability in other disorders. Thus the increase of hypothalamic NPY is an effective therapeutic application against alopecia, early hair loss, subcutaneous fat loss, bone dysfunction and recognition memory.

**Hypothalamic NPY overexpression rescues body weight loss in a mouse model of human premature aging**

Face-1/Zmpste24-/- mice were Face-1/Zmpste24-deficient mice were generated and genotyped as described before (Pendas et al., 2002) and kindly provided by Carlos Lopez-Otin (University of Oviedo, Spain). Mice were housed two per cage under a 12 h light/dark cycle in a
temperature/humidity controlled room with ad libitum access to water and a standard chow diet. Face-1/Zmpste24 −/− mice (2 month old) were randomly divided into two groups: control group (saline-injected Face-1/Zmpste24 −/−) and NPY overexpressing group (AAV-NPY-injected Face-1/Zmpste24 −/−). Recombinant AAV particles were generated as described before (Sousa-Ferreira et al., 2011) Adeno-associated virus (AAV) −1/2 chimerical capsids, containing recombinant plasmids with NPY cDNA under a neuronal specific promoter, were injected in mice hypothalamic arcuate nucleus (ARC), in order to induce constitutive and sustained NPY overexpression. Mice were anesthetized with an intraperitoneal injection of ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively) and placed on a stereotaxic frame. Injection was performed bilaterally into the ARC: 0.5 mm lateral to the middle line, 1.65 mm posterior to the bregma and −5.8 mm ventral to the brain surface. The control group received sterile saline in a final volume of 1.5µl/side. The NPY overexpressing group received 3.6x10⁹v.g./side of AAV-hSyn-NPY, in a final volume of 1.5µl/side. Injection was performed at a rate of 0.5 µl/min with a 10 ml- Hamilton syringe attached to an automatic Pump Controller (WPI). Needle was kept in place for 5 minutes to minimize backflow. Mice were allowed to recover for 2 days. The ARC was defined using The Paxino's Mouse Brain Atlas and AAV infection was anatomically localized by EGFP-expressing neurons in the ARC. Mice were housed in pairs and monitored after AAV injections. Since NPY is a potent orexigenic neuropeptide, to avoid major weight changes, AAV-NPY injected mice were pair-fed (given the same amount of food that AAV-GFP injected mice ate, daily - approximately 4-5 g/day). Each mouse was weighted every other day, for weight
control. Fig. 1 shows that overexpressing NPY in the hypothalamus induces a sustained body weight gain in Face-l/Zmpste24−/− mice. At 2 months of age, Face-l/Zmpste24−/− mice are smaller and have a lower body weight (15.4 ± 0.3 g) compared to age-matched wild type (Zmpste24+/+) littermates (22.8 ± 0.4 g). Thirty days after AAV injection, saline-injected Zmpste24−/− mice gain less weight than the AAV-NPY-injected Face-l/Zmpste24−/− (27.7 ± 0.4 g), which have a similar body weight to age-matched Zmpste24+/+ littermates (26.4 ± 0.6 g). Ninety days after AAV injections, saline-injected Face-l/Zmpste24−/− mice (5 month old) still exhibited a lower body weight (17.7 ± 1.1 g) than AAV-NPY-injected Face-l/Zmpste24−/− mice (27.2 ± 1.7 g).

In conclusion, hypothalamic NPY overexpression rescues body weight loss in this mouse model of human premature aging.

**Hypothalamic NPY overexpression increases fat depots, ameliorating lipodystrophy, in a mouse model of human premature aging**

Adipose tissue, particularly subcutaneous adipose tissue, is reduced in Face-l/Zmpste24−/− mice. Fig. 2 shows that hypothalamic NPY overexpression (as described in example 1) increases adipose tissue mass in Face-l/Zmpste24−/− mice. AAV-NPY-injected Face-l/Zmpste24−/− mice have an increase in both subcutaneous and visceral fat and also gonadal fat depots increase.

In conclusion, hypothalamic NPY overexpression decreases lipodystrophy in a mouse model of human premature aging.

**Hypothalamic NPY overexpression delays alopecia/fur loss in a mouse model of human premature aging**
The Fig. 3 shows that hypothalamic NPY overexpression decreases/delays fur loss in Face-1/Zmpste24−/− mice. Fur loss was assessed twice a week and quantified by a semi-quantitative ranking score system on a scale of 0 to 3: 0 for absent fur loss, 1 for mild fur loss, 2 for moderate fur loss, and 3 for marked fur loss. The results were summed up for each animal group after 30, 60, 90 and 120 days upon hypothalamic injections and the frequency of each ranking score was calculated and plotted on a bar chart. Both saline-injected Face-1/Zmpste24−/− and AAV-NPY-injected Face-1/Zmpste24−/− mice loose fur, however alopecia is more evident in control mice at all time points evaluated. In conclusion, hypothalamic NPY overexpression delays alopecia in a mouse model of human premature aging.

**Hypothalamic NPY overexpression reduces bone fractures risk, the degree of kyphosis of the spine and muscular dystrophy in the mouse model of human premature aging**

Face-1/Zmpste24−/− mice phenotype is characterized by osteoporosis, muscular dystrophy, kyphosis of the spine, a hunched position and scoliosis, reduction of cortical and trabecular bone volumes and develop multiple spontaneous bone fractures particularly ribs fractures. By 6 months of age, nearly every rib in Face-1/Zmpste24−/− mice was broken in the vicinity of the costovertebral junction, showing the formation of hypertrophic calluses at the fracture sites. Face-1/Zmpste24−/− mice with hypothalamic NPY overexpression showed less muscular dystrophy with an increased lower limb muscle mass, compared to saline-injected mice. Face-1/Zmpste24 mice with hypothalamic NPY overexpression have lower degree of kyphosis of the spine in (resulting in a normal posture), a reduced number of rib fractures (demonstrated by the reduced number of hypertrophic
calluses at the costovertebral junctions) when compared with saline-injected Face-l/Zmpste24 /- mice.

In conclusion, hypothalamic NPY overexpression reduces bone fractures risk, the degree of kyphosis of the spine, and muscular dystrophy in the mouse model of human premature aging.

Hypothalamic NPY overexpression improves spatial recognition memory in a mouse model of human premature aging

For the assessment of mice locomotor horizontal activity, open field tests were performed. Mice were acclimated into test room for 60 min. Mice were placed in a 50x50 cm arena with 50 cm high walls and their movement activity was recorded for 30 min using the Acti-Track System (Panlab, Barcelona, Spain). Mean values for total distance travelled were calculated. To assess the memory/cognitive performance in mice, a two-trial Y-maze test designed to measure spatial recognition memory was performed (Dellu et al. 1997). The three arms of the Y-maze were randomly designated: start arm, in which rats started to explore (always open), novel arm, which was blocked during the first trial, but open during the second trial, and other arm (always open). The Y-maze task consisted of two trials separated by an inter-trial interval (ITI) of 2 h. During the first trial (training, 8 min), mice were allowed to explore only two arms (start and other arm), with the third arm (novel arm) closed. For the second trial (after ITI), the mice were placed back in the same starting arm, with free access to all three arms for 8 min. The number of entries and time spent in each arm was determined. Data was expressed as percentage of total entries during the 8 min. Data was also expressed as percentage of time spent in each
time for the total 8 min. Fig. 4 shows that hypothalamic NPY overexpression increases memory performance of Face-l/Zmpste24 −/− mice but does not alter the locomotor activity of these mice. Three months after hypothalamic injection, saline-injected Face-l/Zmpste24 −/− mice show an abnormal posture characterized by a hunched position and scoliosis and mice began to splay their hind paws while walking. Although AAV-NPY- injected Face-l/Zmpste24 −/− mice, that have increased levels of hypothalamic NPY, showed a normal posture, no alterations were found in the locomotor activity of these mice compared to saline-injected Face-l/Zmpste24 −/− mice. However, AAV-NPY Face-l/Zmpste24 −/− mice have a better performance in the Y maze test. AAV-NPY Face-l/Zmpste24 −/− mice spend more time to explore the novel arm than saline-injected mice at all time points studied after AAV injection (30 days: 21%; 60 days: 51%; 90 days: 57%; 120 days: 97%).

This methodology of increasing the endogenous hypothalamic NPY, by using viral vectors, is a new technological approach applied to progeria syndromes (Hutchinson-Gilford syndrome; Werner Syndrome), to deteriorations associated to normal ageing, to aged related pathologies (such as Alzheimer's disease; Parkinson's disease; Machado Joseph disease), to genetic or acquired lipodystrophies (disorders characterized by the loss of body fat), and cancer. Moreover, an increase of endogenous hypothalamic NPY by the methodology described here, by stimulating food intake and adiposity, is a therapeutics strategy to be applied in anorexia and cachexia associated with aging and with other chronic conditions (i.e: cancer, rheumatoid arthritis, HIV infection, and chronic lung, heart, or kidney disease).
Naturally, the present embodiments are not in any way limited to the embodiments described in this document and a person with average knowledge in the field will be able to predict many possible changes to it without deviating from the main idea, as described in the claims.

2. Hypothalamic neuropeptide Y overexpression for use, according to the previous claim, in therapies against progeroid syndromes.

3. Hypothalamic neuropeptide Y overexpression for use, according to any one of the previous claims, wherein the progeroid syndromes is the Hutchinson-Gilford progeria syndrome.

4. Hypothalamic neuropeptide Y overexpression for use, according to claim 1, wherein the aged related pathologies are Alzheimer's disease, Parkinson's disease or Machado Joseph disease.

5. Hypothalamic neuropeptide Y overexpression for use in the treatment of genetic or acquired lipodystrophies.

6. Hypothalamic neuropeptide Y overexpression for use, according to the previous claim, wherein the lipodystrophies are disorders such as selective loss of body fat, anorexia and cachexia.

7. Hypothalamic neuropeptide Y overexpression for use, according to any of the claims 5-6, wherein anorexia and cachexia are associated with other chronic conditions, for example cancer, rheumatoid arthritis, HIV infection, and chronic lung, heart, or kidney disease.
Figure 1
Male

Saline-injected
Face-1/Zmspte24\(^{+/+}\)  AAV-NPY-injected
Face-1/Zmspte24\(^{+/+}\)

Female

Saline-injected
Face-1/Zmspte24\(^{+/+}\)  AAV-NPY-injected
Face-1/Zmspte24\(^{+/+}\)

Figure 2
Figure 3
Figure 4

A

Time after AAV-NPY injection

<table>
<thead>
<tr>
<th>Time</th>
<th>Distance travelled (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td>S</td>
</tr>
<tr>
<td>60 days</td>
<td>S</td>
</tr>
<tr>
<td>90 days</td>
<td>S</td>
</tr>
<tr>
<td>120 days</td>
<td>S</td>
</tr>
</tbody>
</table>

- Saline-injected (S) Face-1/Zmpste24<sup>−/−</sup>
- AAV-NPY-injected (NPY) Face-1/Zmpste24<sup>−/−</sup>

B

Time after AAV-NPY injection

<table>
<thead>
<tr>
<th>Time</th>
<th>Duration of novel arm visits (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td>S</td>
</tr>
<tr>
<td>60 days</td>
<td>S</td>
</tr>
<tr>
<td>90 days</td>
<td>S</td>
</tr>
<tr>
<td>120 days</td>
<td>S</td>
</tr>
</tbody>
</table>

- Saline-injected (S) Face-1/Zmpste24<sup>−/−</sup>
- AAV-NPY-injected (NPY) Face-1/Zmpste24<sup>−/−</sup>

**Figure 4**
**INTERNATIONAL SEARCH REPORT**

International application No

PCT/IB2014/060951

---

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07K14/575 A61K38/22 A61K48/00

---

**B. FIELD SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K C12N

---

**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>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>wo 2010/071454 AI (AUCKLAND UNISERVICES LTD) 24 June 2010 (2010-06-24) pages 1,4; claims 1,6,8, 10, 14-20</td>
<td>1,4</td>
</tr>
<tr>
<td>Y</td>
<td>us 4 891 357 A (KALRA SATYA P) 2 January 1990 (1990-01-02) claims 1-14 column 4, line 30 - line 39</td>
<td>1,5-7</td>
</tr>
<tr>
<td>Y</td>
<td>wo 2005/037211 A2 (NEUROLOGIX RES INC) 28 April 2005 (2005-04-28) page 5, last paragraph page 29, last paragraph - page 34, paragraph 2 example 1 claims 1-56</td>
<td>1,5-7</td>
</tr>
</tbody>
</table>

---

**Date of the actual completion of the international search**

5 August 2014

**Date of mailing of the international search report**

20/08/2014

---

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HN Rijswijk

Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

**Authorized officer**

Mandl, Birgit

---

* Special categories of cited documents:

**A** document defining the general state of the art which is not considered to be of particular relevance

**E** earlier application or patent but published on or after the international filing date

**L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

**O** document referring to an oral disclosure, use, exhibition or other means

**P** document published prior to the international filing date but later than the priority date claimed

---

* Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

* Document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

* Document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

* Document member of the same patent family
<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>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FENPING ZHENG ET AL: &quot;Overexpression of Neuropeptide Y in the Dorsomedial Hypothalamus Causes Hyperphagia and Obesity&quot;, OBESITY, vol. 21, no. 6, 21 March 2013 (2013-03-21), pages 1086-1092, XP055132936, ISSN: 1930-7381, DOI: 10.1002/oby.20467 abstract page 1087, left-hand column, paragraph 2 -----</td>
<td>1-8</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>WO 2010071454 A1</td>
<td>24-06-2010</td>
<td>NONE</td>
</tr>
<tr>
<td>US 4891357 A</td>
<td>02-01-1990</td>
<td>NONE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2542417 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1680145 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2008505602 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2005136036 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2010168215 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2005037211 A2</td>
</tr>
</tbody>
</table>