COMPOSITION FOR USE IN THE TREATMENT OF NEURODEGENERATIVE DISEASES WITH PARKINSONIAN SYNDROMES

The present invention relates to a composition comprising an inhibitor of the COMT enzyme for use in the treatment of a Neurodegenerative Disease with Parkinsonian Syndromes in a subject having a COMT allele, wherein said composition does not comprise L-Dopa.

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COMPOSITION FOR USE IN THE TREATMENT OF NEURODEGENERATIVE DISEASES WITH PARKINSONIAN SYNDROMES

FIELD OF INVENTION

The present invention relates to the treatment of Neurodegenerative diseases with Parkinsonian Syndromes in subjects in need thereof. More particularly, the present invention relates to a composition for treating motor symptoms related to Neurodegenerative Diseases with Parkinsonian Syndromes. The composition of the invention comprises an inhibitor of the COMT enzyme, and is of particular interest for subjects with a COMT<sup>H</sup> allele.

BACKGROUND OF INVENTION

Degenerative Diseases with Parkinsonian Syndromes such as Parkinson Disease comprise motor symptoms and non-motor symptoms. The motor symptoms of degenerative disorders involving the dopaminergic system such as Parkinson Disease are collectively called "Parkinsonian Syndromes". Motor symptoms include, without limitation, bradykinesia, tremor at rest, rigidity or stiffness, shaking, slowness of movement and postural instability. Idiopathic Parkinson Disease is the most common cause of Parkinsonian Syndrome (about 65%). Other causes are Progressive Supranuclear Palsy, Multiple System Atrophy, Corticobasal Degeneration and Lewy Body Dementia.

Non-motor symptoms may include autonomic dysfunction, cognitive (impairment of cognitive and executive performances) and behavioral problems leading sometimes to dementia, and sensory, sleep and emotional problems (mostly depression). Treatment of these non-motor symptoms is not yet standardized although some drugs have been proposed such as antidepressant drugs (depression), clozapine (illusions,
hallucinations), cholinesterase inhibitors (dementia treatment) and modafinil (sleep problems treatment).

Neurodegenerative diseases with Parkinsonian Syndromes are characterized by the loss of pigmented dopaminergic neurons in the mesencephalon.

Therefore, pharmacotherapy has focused on dopaminergic drugs, mainly the dopamine precursor, L-dopa (also known as Levodopa), and dopamine receptor agonists. L-dopa is transformed to dopamine by the aromatic amino acid decarboxylase (AADC). Conversion of L-dopa to dopamine in the periphery is disadvantageous as dopamine does not cross the blood brain barrier into the central nervous system (CNS). L-dopa is thus usually administered in combination with AADC inhibitors (such as carbidopa and benserazide) which help to prevent the metabolism of L-dopa in the periphery before it reaches the CNS. In the CNS, two main enzymes, monoamine oxidase B (MAO-B) and catechol-O-methyltransferase (COMT) metabolize the dopamine into other by-products.

However, as the disease progresses, patients experience periods during which they have parkinsonian symptoms (OFF state) and periods during which symptoms are effectively alleviated by the treatment (ON state). Although this phenomenon of motor fluctuation in Parkinson Disease is not fully understood, the elimination of L-dopa from plasma in combination with the progression of the dopaminergic denervation seems to play an important role. Because L-dopa is systematically administered with AADC inhibitors, its metabolism in periphery is switch towards the COMT pathway. Co-administration of COMT inhibitors, such as entacapone or tolcapone, has demonstrated benefit on motor fluctuations by reducing the OFF periods and increasing the ON periods. COMT inhibitors are thus commonly used in Parkinson Disease patients with motor fluctuations in adjunction to L-dopa. In addition to the motor fluctuations, long-term administration of L-Dopa induces abnormal movement, as the L-dopa-induced dyskinesia.

Treatments with inhibitors of COMT (Tolcapone) alone were tested in early Parkinson Disease patients, but researchers failed to identify any symptomatic benefit of said treatment (Hauser et al, Movement Disorders, 1998, 13(4): 643-7). This was further explained by the assumption that the role of COMT is much more important in the
prefrontal cortex (region of the brain involved in cognitive impairment associated with Parkinson Disease) than in the striatum (region involved in motor dysfunction) (Tunbridge et al, Biol Psychiatry, 2006, 60:141-51).

As a result of these observations, an effect on an inhibitor of the COMT enzyme alone on Parkinsonian Syndromes in the general population appeared highly unlikely.

The distribution of the COMT enzyme in the population and its segregation in families indicate that it is regulated by a single autosomal locus with 2 co-dominant alleles. The substitution of valine by methionine at codon 158 in the membrane-bound isoform of COMT results in a trimodal distribution of high (valine/valine, referred as H/H), intermediate (valine/methionine, referred as H/L) and low (methionine/methionine, referred as L/L) enzymatic activity.

The impact of COMT genotype on the risk for a patient to develop Parkinson Disease or another Neurodegenerative Disease with Parkinsonian Syndromes is currently controversial. Indeed, some have suggested that the COMT\(^{L/L}\) genotype is a risk factor for Parkinson Disease (Bonifati et al, Pharmacol. Ther., 1999, 81(1):1-36), whereas others identified no difference in COMT allele frequencies between Parkinson Disease patients and healthy controls (Lee et al., Neuroscience Letters, 2001, 298:131-4).

Moreover, as for motor functions, some experimental results seem to show that there is no impact of the COMT polymorphism on motor dysfunction associated with Parkinson Disease (Contin et al, Movement Disorder, 2005, 20(6):734-51).

Here, the inventors showed that, surprisingly, the polymorphism of the COMT gene directly impacts the age of onset of motor symptoms in Parkinson Disease patients. Indeed, the inventors showed that said onset occurred with a median of 2 years earlier in patients with high or intermediate COMT activity (COMT\(^{H/H}\)) and COMT\(^{A}\) patients) than in patients with low COMT activity (COMT\(^{A}\) patients).

The aim of the present invention is therefore to administer a COMT inhibitor without L-dopa to treat Neurodegenerative Diseases with Parkinsonian Syndromes in COMT\(^{H/H}\) or COMT\(^{H/L}\) subjects, in order to treat motor symptoms. In addition, early treatment with a COMT inhibitor without L-dopa would postpone L-dopa treatment in COMT\(^{H/H}\) or COMT\(^{H/L}\) subjects, thus avoiding its drawbacks and in particular limiting the development of motor complications (fluctuations and dyskinesia).
SUMMARY

The present invention thus relates to a composition comprising an inhibitor of the COMT enzyme for use in the treatment of a Neurodegenerative Disease with Parkinsonian Syndromes in a subject having a COMT allele, wherein said composition does not comprise L-Dopa.

In one embodiment of the invention, the inhibitor of COMT is an inhibitor of COMT activity. In one embodiment, said inhibitor of COMT activity is selected from the group comprising, nitecapone, 2-(3,4-dihydroxy-2-nitrophenyl)vinyl ketone, entacapone, dihydroxynitrobenzaldehyde, tolcapone, 6-nitronoradrenaline, dinitrocatechol, 1,2-dihydroxyl-3-hydroxypyridine-4-one; vinylphenylketone; CGP 28014; 1,2-dimethyl-3-hydroxypyridine-4-one, preferably said inhibitor of COMT activity is entacapone or tolcapone.

In one embodiment of the invention, the inhibitor of COMT is an inhibitor of the expression of the gene encoding the COMT enzyme. In one embodiment, said inhibitor of the expression encoding the COMT enzyme is selected from the group comprising siRNAs, shRNAs, antisense oligonucleotide, ribozymes or aptamers of COMT.

In one embodiment of the invention, the patient is COMT or COMT.

In one embodiment of the invention, the Neurodegenerative Disease with Parkinsonian Syndromes is Parkinson disease, progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration or Lewy body dementia.

In one embodiment of the invention, motor symptoms related to the Neurodegenerative Disease with Parkinsonian Syndromes are to be treated. In one embodiment of the invention, the motor symptom to be treated is selected in the group comprising tremor at rest; akinesia and rigidity, such as, for example, slowness of movements, amimia, micrographia, loss of arm swing, difficulties in walking, sensation of stiffness; joint pain, dystonia, swallowing disorders, abnormal tiredness, trembling sensation, bradykinesia, action tremor, tremors, dysarthria, dysautonomia, dysphagia, dystonia, eye apraxia, limb apraxia, myoclonus, oculomotor tremors and night tremor.
In one embodiment of the invention, the subject is at risk of developing a Neurodegenerative Disease with Parkinsonian Syndromes. In one embodiment of the invention, the subject is diagnosed with a Neurodegenerative Disease with Parkinsonian Syndromes.

DETAILED DESCRIPTION

The present invention relates to a composition comprising a COMT inhibitor for treating a Neurodegenerative Disease with Parkinsonian Syndromes in a subject having a COMT\textsuperscript{H} allele. The present invention also relates to a composition comprising a COMT inhibitor for use in the treatment of a Neurodegenerative Disease with Parkinsonian Syndromes in a subject having a COMT\textsuperscript{H} allele.

In one embodiment, the composition for use of the invention does not comprise L-dopa. In one embodiment of the invention, the composition of the invention consists of a COMT inhibitor.

As used herein, the term "Parkinsonian syndrome" relates to a vast number of motor symptoms which are usually associated with Degenerative Disorders involving the dopaminergic system such as Parkinson Disease. Symptoms of a parkinsonian syndrome may include, without limitation, tremor at rest; akinesia and rigidity, such as, for example, slowness of movements, animia, micrographia, loss of arm swing, difficulties in walking, sensation of stiffness; joint pain, dystonia, swallowing disorders, abnormal tiredness, trembling sensation, bradykinesia, action tremor, tremors, dysarthria, dysautonomia, dysphagia, dystonia, eye apraxia, limb apraxia, myoclonus, oculomotor tremors, night tremor, gait and posture impairment, sleep disorders. ..

In one embodiment, said parkinsonian syndrome is a degenerative parkinsonian syndrome or an irreversible secondary parkinsonian syndrome.

Examples of Neurodegenerative Disease with Parkinsonian Syndromes include, but are not limited to, Parkinson disease, progressive supranuclear palsy, multiple system
atrophy, corticobasal degeneration or Lewy body dementia. Preferably, the Neurodegenerative Disease with Parkinsonian Syndromes is Parkinson disease, more preferably an idiopathic form of Parkinson disease.

In one embodiment, the Neurodegenerative Disease with Parkinsonian Syndromes to be treated is Parkinson disease, such as for example idiopathic or genetic form of Parkinson disease.

In one embodiment, the Neurodegenerative Disease with Parkinsonian Syndromes to be treated is progressive supranuclear palsy.

In one embodiment, the Neurodegenerative Disease with Parkinsonian Syndromes to be treated is multiple system atrophy.

In one embodiment, the Neurodegenerative Disease with Parkinsonian Syndromes to be treated is corticobasal degeneration.

In one embodiment, the Neurodegenerative Disease with Parkinsonian Syndromes to be treated is Lewy body dementia.

As used herein, the term "treatment" refers to preventing (i.e. keeping from happening), reducing or alleviating at least one adverse effect or symptom of a disease, disorder or condition associated with a deficiency in or absence of an organ, tissue or cell function.

In one embodiment, the composition for use of the invention is for preventing, reducing or alleviating the motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. In one embodiment, the alleviation or reduction of a symptom corresponds to a diminution of the number of occurrence of said symptom per day. For example, the alleviation of tremors may correspond to a decrease in the number of crisis, or the total duration of tremors per day. In another embodiment, the alleviation or reduction of said symptoms may also correspond to a decrease in the intensity of said symptom. For example, the alleviation of tremors may correspond to a decrease in the intensity of the crisis of tremors. In a preferred embodiment, the decrease or alleviation of a symptom corresponds to both a decrease in the number of occurrence of said symptom and in a decrease in the intensity of said symptom.
Examples of motor-symptoms which may be prevented, reduced and/or alleviated include, but are not limited to, tremor at rest; akinesia and rigidity, such as, for example, slowness of movements, animia, micrographia, loss of arm swing, difficulties in walking, sensation of stiffness; joint pain, dystonia, swallowing disorders, abnormal tiredness, trembling sensation, bradykinesia, action tremor, tremors, dysarthria, dysautonomia, dysphagia, dystonia, eye apraxia, limb apraxia, myoclonus, oculomotor tremors, night tremor...

The skilled artisan, who is a specialist of the treatment of a Neurodegenerative Disease with Parkinsonian Syndromes, knows how to evaluate the efficacy of a treatment of a Neurodegenerative Disease with Parkinsonian Syndromes, preferably to evaluate the reduction or alleviation of the motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. For example, scales exist to assess the severity of the motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. Examples of scales which may be used to assess the efficacy of the composition of the invention on the treatment of Neurodegenerative Disease with Parkinsonian Syndromes, preferably on motor symptoms include, but are not limited to, The Unified Parkinson's Disease Rating Scale (UPDRS) in its old or its new version (MDS-UPDRS), preferably section III (which is specific of motor examination); dyskinesia scales, such as, for example, Goetz, CAPIT, CAPSIT or Marconi scales, as well as MDS-UDysRS scale; dysarthria scales; swallowing scales; ...

In one embodiment, the composition for use of the invention may also be for preventing, reducing or alleviating the non-motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. Examples of non-motor symptoms which may be prevented, reduced and/or alleviated include, but are not limited to, autonomic dysfunction, impairment of cognitive performance, impairment of executive performances, behavioral problems, such as, for example, behavioral problems leading to dementia, sensory problems, sleep problems, emotional problems such as, for example, depression...

The skilled artisan knows how to evaluate the efficacy of a treatment of a Neurodegenerative Disease with Parkinsonian Syndromes, preferably to evaluate the
reduction or alleviation of the non-motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. For example, scales exist to assess the severity of the non-motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. Examples of scales which may be used to assess the efficacy of the composition of the invention on the treatment of Neurodegenerative Disease with Parkinsonian Syndromes, preferably on non-motor symptoms include, but are not limited to, The Unified Parkinson's Disease Rating Scale (UPDRS), preferably sections I, II and VI; Neuropsychological scales such as, for example, MMS and BREF scales; Mood evaluation scales, such as, for example, Hamilton scale and MADRS scale and Quality-of-life scales, such as, for example, Goetz, CAPrf, CAPSIT, and Marconi scales.

As used herein, the initials "COMT" stands for the Catechol-O-methyl transferase enzyme.

The COMT enzyme, having the Enzyme Classification number EC 2.1.1.6, catalyzes the O-methylation of catecholamines and other catechols. The enzyme catalyzes the introduction of a methyl group to the catecholamine, which is donated by S-adenosyl methionine (SAM). Any compound having a catechol structure, like catecholestrogens and catechol-containing flavonoids, are substrates of COMT.

According to the invention, the inhibitor of COMT may be an inhibitor of COMT activity or an inhibitor of the expression of the gene encoding the COMT enzyme. Preferably, the inhibitor of COMT is an inhibitor of COMT activity.

In one embodiment of the invention, the inhibitor of COMT is an inhibitor of COMT activity. According to the invention, an "inhibitor of COMT activity" refers to a natural or synthetic compound that decreases or prevents the enzymatic activity of the COMT enzyme.

Examples of inhibitor of COMT activity which may be used in the composition of the present invention include, but are not limited to, nitrocatechols, such as nitecapone (OR-
462; 3-(3,4-dihydroxy-5-nitro-benzylidene)-2,4-pentanedione), 2-(3,4-dihydroxy-2-nitrophenoxy)vinyl ketone, entacapone (OR-611; (E)-2-cyano-N,N-diethyl-3-(3,4-dihydroxy-5-nitrocinnamamide)), dihydroxynitrobenzaldehyde, tolcapone (RO 40-7592; 4'-methyl-3,4-dihydroxy-5-nitro-benzophenone), 6-nitronoradrenaline (also referred as 6-nitronorepinephrine), dinitrocatechol (OR-486), 1,2-dihydroxyl-3-hydroxypyridine-4-one; vinylphenylketone (QO IIIR; 2-(3,4-dihydroxy-2-nitrophenyl)vinyl phenyl ketone); hydroxypyridine compounds such as, for example, CGP 28014; active iron chelators such as, for example, 1,2-dimethyl-3-hydroxypyridine-4-one.

In another embodiment of the invention, the inhibitor of COMT is an inhibitor of COMT expression. As used herein, an "inhibitor of COMT expression" refers to a natural or synthetic compound that has a biological effect to inhibit or significantly decrease the expression of the gene encoding the COMT enzyme.

Examples of inhibitors of COMT expression include, but are not limited to, siRNAs, shRNAs, antisense oligonucleotide, ribozymes or aptamers of COMT.

In one embodiment, the COMT inhibitor present in the composition for use of the invention is capable to cross the blood brain barrier (BBB) into the central nervous system. Examples of COMT inhibitor capable to cross the BBB include, but are not limited to, entacapone and tolcapone. Without willing to be linked to a theory, the Applicant suggests that a COMT inhibitor capable to cross the BBB may increase dopamine bioavailability in the brain and thus have therapeutic consequences on Neurodegenerative Diseases with Parkinsonian Syndromes, even without L-dopa.

The COMT gene (SEQ ID NO: 1, Accession Number Z26491), localized in humans on chromosome 22, comprises two different initiation sites for transcription, and encodes both a cytosolic (SEQ ID NO: 2, NCBI reference sequence NP_009294.1) and a membrane-bound form (SEQ ID NO: 3, NCBI reference sequence NP_000745; corresponding to the cDNA sequence of SEQ ID NO: 4, Accession Number
NM_000754.3) of the enzyme. In the brain, the membrane-bound form is highly expressed (with a ratio of membrane-bound / cytosolic of about 70/30.

As shown herein after, the sequence of the membrane-bound form of the COMT enzyme includes an additional 50 amino acids segment at the N-terminus (italicized) that is not present in the cytosolic form of the enzyme.

\[
\begin{align*}
\text{MPEAPPPLLAAVLLGLVLLVVLLLRR} & \quad \text{WHGWGLCLIGWNE} \\
\text{FILQP1HNLILMGDTKEQRILNHVLQHAEPGANQSVELAID} \\
\text{TYCEQKEWAMNVGDKKGKVDAVIEHQPSVLLLLGAY} \\
\text{CGYS AVRMARLLSPGARLITEINPDCIAITQRMVDFAG} \\
\text{VKDKVTLVVGA SQDIIPQLK KKYD VDTLD MVLFDH WKD} \\
\text{RYLPDTLLL LEECGLLRKGTVLLADVINCPGAPDFL AHV R} \\
\text{GSCFECTHY QS FLEYREV DVDG LKAI YKGPG SEAGP}
\end{align*}
\]

In one embodiment, the COMT inhibitor present in the composition for use of the invention inhibits both the cytosolic and membrane-bound isoforms of the COMT enzyme.

A single nucleotide polymorphism (SNP) in the COMT gene causes a trimodal distribution of low, intermediate and high activity. That polymorphism is caused by autosomal codominant alleles and results from a single G to A transition, leading to a change in amino acid from valine to methionine at codon 108 of the cytosolic form (or 158 of the membrane-bound form). The SNP polymorphism may be referred as \textit{rs4680} or G158A or Vall58Met polymorphism. The position of the Vall58Met polymorphism is highlighted in bold underscored in the sequence shown herein above.

The low activity form of the enzyme, referred as "L", has a A nucleotide and thus a Methionine at that position; whereas the high activity form, referred as "H", has a G and thus a Valine at that position.

Subjects with low COMT activity possess two COMT\textsuperscript{L} alleles, and may be referred as COMT\textsuperscript{L} subjects. Subjects with high COMT activity possess two COMT\textsuperscript{H} alleles, and may be referred as COMT\textsuperscript{H} subjects. Subjects with intermediate COMT activity.
possess one COMT<sup>L</sup> allele and one COMT<sup>H</sup> allele, and may be referred as COMT<sup>H/L</sup> subjects.

The Inventor showed that high metabolizers (COMT<sup>H/H</sup>) have an earlier age at PD onset than low metabolizers (COMT<sup>L/L</sup>) (Example 1). Without willing to be linked by a theory, the Inventor suggests that COMT activity modulate dopamine biodisponibility in the brain and that low COMT activity may be one of the compensatory mechanisms at early stage of the disease by maintaining a high level of dopamine in the brain despite striatal DA denervation. Therefore, the Inventor suggests that the administration of a COMT inhibitor alone to high or intermediate metabolizers may treat a Neurodegenerative Disease with Parkinsonian Syndromes, particularly in the early stage of the disease.

As used herein, the term "early stage of the disease" means during the first years after the diagnosis of said disease, before the occurrence of motor fluctuations. Depending of disease severity in an individual patient or disease subtype, the term "early stage of the disease" can thus mean several years of disease duration. In one embodiment, the term "early stage of the disease" means the first year, the first two, three, four, five, six, seven, eight, nine or ten years after the diagnosis of the disease.

As used herein, a subject is a mammal, preferably a human. In one embodiment, the subject is a male. In another embodiment, the subject is a female.

In one embodiment of the invention, the composition for use of the present invention is for treating, or for use in the treatment of, a Neurodegenerative Disease with Parkinsonian Syndromes in a COMT<sup>H/H</sup> subject.

In one embodiment of the invention, the composition for use of the present invention is for treating, or for use in the treatment of, a Neurodegenerative Disease with Parkinsonian Syndromes in a COMT<sup>H/L</sup> subject.

The determination of the COMT genotype of a subject may be carried out by methods known to the skilled artisan. Said methods may be carried out on a cell sample obtained
from the subject, such as for example, a blood sample or a sample obtained after a biopsy has been carried out on the subject.

Some genotyping methods which may be used in the present invention are described for example in US2003/0100476, which is incorporated herein by reference. Examples of such methods include, but are not limited to, PCR-based restriction fragments length polymorphism analysis using the restriction enzyme \( N_{al}III \), allele specific hybridization, use of a primer in a polymerase chain reaction (PCR), such as, for example, anchor PCR or RACE PCR or in a ligase chain reaction (LCR), identification of alterations in restriction enzyme cleavage pattern, sequencing reactions, analysis of the protection from cleavage agents (such as, for example, nuclease, hydroxylamine or osmium tetroxide and with piperidine), recognition of mismatched base pairs in double strand DNA by specific enzymes, alterations in electrophoretic mobility, analysis of the movement of polymorphic fragments in polyacrylamide gels containing gradients of denaturant (denaturing gradient gel electrophoresis, DGGE), selective oligonucleotide hybridization (for example using a specialized exonuclease-resistance nucleotide), selective amplification depending on selective PCR or selective primer extension, oligonucleotide ligation assays, expansion methods using dideoxynucleotides derivatives, Genetic Bit Analysis (GBA™), in situ detection using an antibody specific of a variant sequence, immunoassays such as, for example, EIA or ELISA, immunofluorescence and the like...

In one embodiment, the genotype determination is carried out as follows:
Genomic DNA was extracted by standard methods from venous blood samples. The COMT polymorphism G158A was analyzed by an allelic discrimination Taqman assay (ABI PRISM 7900 sequence detection system) (Applied Biosystems, Forster City, USA) that uses the 50-nuclease activity of Taq DNA polymerase to detect a fluorescent reporter signal generated after the polymerase chain reaction (Assay ID: C_25746809_50, Applied Biosystem).

In one embodiment of the invention, the subject has been diagnosed with a Neurodegenerative Disease with Parkinsonian Syndromes. Preferably, said Neurodegenerative Disease with Parkinsonian Syndromes is selected from the list
comprising Parkinson disease, progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration or Lewy body dementia.

Methods for diagnosing a Neurodegenerative Disease with Parkinsonian Syndromes are well-known to the skilled artisan. Examples of suitable methods include, but are not limited to, assessment of the presentation of a Parkinsonian Syndrome (including the presentation of one or more of the following symptoms: tremor at rest, akinesia, rigidity, depression, joint pain, dystonia, anosmia, swallowing disorders, abnormal tiredness, trembling sensation, levodopa response...); Neuroimaging; Functional cerebral imaginal by PET, DAT scan.

In one embodiment of the invention, the subject has been diagnosed with a Neurodegenerative Disease with Parkinsonian Syndromes since less than 10 years, 9, 8, 7, 6, 5, 4, 3 years, preferably less than 2 years, more preferably less than 1 year.

In one embodiment of the invention, the subject is at risk of developing a Neurodegenerative Disease with Parkinsonian Syndromes.

In one embodiment of the invention, the subject has a genetic or familial predisposition to a Neurodegenerative Disease with Parkinsonian Syndromes.

In one embodiment of the invention, the subject presents a non-genetic predisposition to a Neurodegenerative Disease with Parkinsonian Syndromes. Non-genetic risk factors for developing a Neurodegenerative Disease with Parkinsonian Syndromes include, but are not limited to, exposure to heavy metals, such as, for example, Lead, Manganese or Copper; exposure to pesticides such as, for example, rotenone or paraquat; exposure to pollutants; exposure to herbicides such as, for example, Substance Orange; exposure to toxic substances, such as, for example, MPTP...

In one embodiment of the invention, the composition for use of the invention further comprises another therapeutic agent useful for treating a Neurodegenerative Disease with Parkinsonian Syndromes. Examples of therapeutic agents include, but are not limited to dopamine agonists, such as, for example, bromocriptine, cabergoline, pergolide, pramipexole, fenoldopam, ropinirole, rotigotine, quinagolide and apomorphine; monoamine oxidase inhibitors, such as, for example, benmoxin,
hydralazine, iproclozide, iproniazid, isocarboxazid, isoniazid, mebanazine, nialamide, octamoxin, phenelzine, pheniprazine, phenoxypropazine, pivalylbenzhydrazine, Pivalylbenzhydrazine, Procarbazine, Safrazine, Caroxazone, Echinopsidine, Furazolidone, Linezolid, Tranylcypromine, Brofaromine, Metralindole, Minaprine, Moclobemide, Pirindole, Toloxatone, Lazabemide, Pargyline, Rasagiline, Selegiline; or other drugs with antiparkinsonian effects other than levodopa, for example methylphenidate, anticholinergic drugs.

The present invention also relates to a pharmaceutical composition for treating a Neurodegenerative Disease with Parkinsonian Syndromes in a subject in need thereof, comprising the composition for use as hereinabove described in combination with at least one pharmaceutically acceptable excipient.

The present invention also relates to a medicament for treating a Neurodegenerative Disease with Parkinsonian Syndromes in a subject in need thereof, comprising the composition for use as hereinabove described.

As used herein, the term "pharmaceutically acceptable" refers to compounds and compositions which may be administered to mammals without undue toxicity. Accordingly, a "Pharmaceutically acceptable excipient" refers to an excipient that does not produce an adverse, allergic or other untoward reaction when administered to an animal, preferably a human. It includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologies standards. Suitable excipients include water, saline, Ringer's solution, dextrose solution, and solutions of ethanol, glucose, sucrose, dextran, mannose, mannitol, sorbitol, polyethylene glycol (PEG), phosphate, acetate, gelatin, collagen, Carbopol®, vegetable oils, and the like. One may additionally include suitable preservatives, stabilizers, antioxidants, antimicrobials, and buffering agents, such as, for example, BHA, BHT, citric acid, ascorbic acid, tetracycline, and the like.
Other examples of pharmaceutically acceptable excipients that may be used in the composition of the invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

In one embodiment, the composition of the invention may comprise some excipients, such as, for example, surfactants (e.g. hydroxypropylcellulose); suitable carriers, such as, for example, solvents and dispersion media containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils, such as, for example, peanut oil and sesame oil; isotonic agents, such as, for example, sugars or sodium chloride; coating agents, such as, for example, lecithin; agents delaying absorption, such as, for example, aluminum monostearate and gelatin; preservatives, such as, for example, benzalkonium chloride, benzethonium chloride, chlorobutanol, thimerosal and the like; buffers, such as, for example, boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like; tonicity agents, such as, for example, dextran 40, dextran 70, dextrose, glycerin, potassium chloride, propylene glycol, sodium chloride; antioxidants and stabilizers, such as, for example, sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like; nonionic wetting or clarifying agents, such as, for example, polysorbate 80, polysorbate 20, poloxamer 282 and tyloxapol; viscosity modifying agents, such as, for example dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxymethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose; and the like.
According to an embodiment, the composition for use, the pharmaceutical composition or the medicament of the invention is injected, preferably systemically injected. Examples of formulations adapted to systemic injections include, but are not limited to, liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. Examples of systemic injections include, but are not limited to, intravenous, subcutaneous, intramuscular, intradermal and intraperitoneal injection, and perfusion. According to an embodiment, when injected, the composition for use, the pharmaceutical composition or the medicament of the invention is sterile. Methods for obtaining a sterile pharmaceutical composition include, but are not limited to, GMP synthesis (GMP stands for "Good manufacturing practice").

According to another embodiment, the composition for use, the pharmaceutical composition or the medicament of the invention is orally administered. Examples of formulations adapted to oral administration include, but are not limited to, solid forms, liquid forms and gels. Examples of solid forms adapted to oral administration include, but are not limited to, pill, tablet, capsule, soft gelatin capsule, hard gelatin capsule, caplet, compressed tablet, cachet, wafer, sugar-coated pill, sugar coated tablet, orodispersing/orodisintegrating tablet, powder, solid forms suitable for solution in, or suspension in, liquid prior to oral administration and effervescent tablet. Examples of liquid form adapted to oral administration include, but are not limited to, solutions, suspensions, drinkable solutions, elixirs, sealed phial, potion, drench, syrup and liquor.

Other examples of administration routes include, but are not limited to, nasal, buccal, rectal, vaginal, topical, intratracheal, endoscopic, transdermal, transmucosal, and percutaneous administration or administration using an aerosol.

In one embodiment of the invention, the composition for use of the invention is administered in a therapeutically effective amount in a subject in need thereof. As used herein, a "therapeutically effective amount" is the amount of a therapeutic agent necessary and sufficient for slowing down or stopping the progression, aggravation, or deterioration of one or more symptoms of the disease, or condition; alleviating the symptoms of the disease or condition; curing the disease or condition.
In one embodiment of the invention, from about 1 to about 5000 mg of the composition for use of the invention are administered to the subject, preferably from about 50 to about 500 mg, more preferably about 100 mg.

In one embodiment of the invention, the composition for use of the invention is administered to the subject at least once a day, preferably at least twice a day, more preferably at least 3 times a day.

In one embodiment of the invention, the composition of the invention is administered to the subject in a daily amount ranging from about 1 to about 15 000 mg, preferably ranging from about 50 to about 1500 mg, more preferably from about 100 to about 300 mg and even more preferably in an amount of about 300 mg.

As used herein, the term "about" preceding a figure means plus or less 10% of the value of said figure.

The present invention thus relates to a composition for treating, or for use in the treatment of, a Neurodegenerative Disease with Parkinsonian Syndromes in a subject with a COMT^H^ allele.

In one embodiment of the invention, the composition is for treating motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes. In another embodiment, the composition may also be for treating non-motor symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes.

In one embodiment of the invention, the composition is for postponing L-dopa treatment in COMT^{H/L} and/or in COMT^{H/L} patients diagnosed with a Neurodegenerative Disease with Parkinsonian Syndromes, preferably in the early stages of the Disease, such as, for example, during the first year, the first 2 years or the first 3 years after diagnosis.

In one embodiment of the invention, the composition is for treating and/or for preventing, symptoms associated with a Neurodegenerative Disease with Parkinsonian Syndromes in a COMT^{H/L} and/or in COMT^{H/L} subject. In one embodiment of the
invention, said subject has no onset of a Neurodegenerative Disease with Parkinsonian Syndromes. In one embodiment, said subject is at risk of developing a Neurodegenerative Disease with Parkinsonian Syndromes.

The present invention also relates to a method for treating a Neurodegenerative Disease with Parkinsonian Syndromes in a subject with a COMT\textsuperscript{H} allele (i.e. a COMT\textsuperscript{H/H} or a COMT\textsuperscript{H/L} subject), wherein said method comprises the administration of therapeutically effective amount of an inhibitor of COMT. In one embodiment of the invention, the method of the invention does not comprise the administration of L-dopa.

In one embodiment, the method of the invention is for treating a motor symptom associated with a Neurodegenerative Disease with Parkinsonian Syndromes. Examples of such motor symptoms include, but are not limited to, tremor at rest; akinesia and rigidity, such as, for example, slowness of movements, amimia, micrographia, loss of arm swing, difficulties in walking, sensation of stiffness; joint pain, dystonia, swallowing disorders, abnormal tiredness, trembling sensation, bradykinesia, action tremor, tremors, dysarthria, dysautonomia, dysphagia, dystonia, eye apraxia, limb apraxia, myoclonus, oculomotor tremors, night tremor...

In one embodiment, the method of the invention is for treating a non-motor symptom associated with a Neurodegenerative Disease with Parkinsonian Syndromes. Examples of such non-motor symptoms include, but are not limited to, autonomic dysfunction, impairment of cognitive performance, impairment of executive performances, behavioral problems, such as, for example, behavioral problems leading to dementia, sensory problems, sleep problems, emotional problems such as, for example, depression...

In one embodiment of the invention, the subject is at risk of developing a Neurodegenerative Disease with Parkinsonian Syndromes. In one embodiment of the invention, the subject has a genetic or familial predisposition to a Neurodegenerative Disease with Parkinsonian Syndromes.
In one embodiment of the invention, the subject presents a non-genetic predisposition to a Neurodegenerative Disease with Parkinsonian Syndromes. Non-genetic risk factors for developing a Neurodegenerative Disease with Parkinsonian Syndromes include, but are not limited to, exposure to heavy metals, such as, for example, Lead, Manganese or Copper; exposure to pesticides such as, for example, rotenone or paraquat; exposure to pollutants; exposure to herbicides such as, for example, Substance Orange; exposure to toxic substances, such as, for example, MPTP...

According to an embodiment, the method of the invention comprises the injection, preferably the systemic injection, of a COMT inhibitor.

According to another embodiment, the method of the invention comprises the oral administration of a COMT inhibitor.

Other examples of administration routes of the COMT inhibitor include, but are not limited to, nasal, buccal, rectal, vaginal, topical, intratracheal, endoscopic, transdermal, transmucosal, and percutaneous administration or administration using an aerosol.

In one embodiment of the invention, the method of the invention comprises the administration of 1 to 5000 mg of a COMT inhibitor, preferably from 50 to 500 mg, more preferably about 100 mg.

In one embodiment of the invention, the COMT inhibitor is administered at least once a day, preferably at least twice a day, more preferably at least 3 times a day.

In one embodiment of the invention, the method of the invention comprises the administration to the subject of a daily amount of the COMT inhibitor ranging from about 1 to about 15 000 mg, preferably ranging from about 50 to about 1500 mg, more preferably from about 100 to about 300 mg and even more preferably in an amount of about 300 mg.

The administration of a COMT inhibitor without co-administration of L-dopa to COMT^{H/H} or COMT^{H/L} subjects presents the following advantages:

- treatment of Neurodegenerative Diseases with Parkinsonian Syndromes, particularly of motor symptoms;
- postponement of the treatment with L-dopa in order to avoid drawbacks associated with the treatment with L-dopa, such as, for example, motor complications (fluctuations and dyskinesia);
- postponement of drawbacks associated with the treatment with L-dopa, such as, for example, motor complications (fluctuations and dyskinesia);
- limitation of the development of motor complications associated with the treatment with L-dopa, such as fluctuations and dyskinesia.

**BRIEF DESCRIPTION OF THE DRAWINGS**

*Figure 1* is a graph showing the survival distributions of AAO estimated using the Kaplan-Meier method. P-values were determined by testing the relationships between AAO and the COMT genotype assuming a dominant model using the log-rank test.

*Figure 2* is a graph showing FRS in mice treated with escalating doses of MPTP at 4, 8, and 16mg/kg/day at J0-J5, J7-J11, J14-J18 respectively, and tolcapone (squares) 10 mg/kg or vehicle (circles), n = 4 per group. Repeated measure ANOVA, F(3,57)=4.6, p =0.006.

**EXAMPLES**

The present invention is further illustrated by the following examples.

**Example 1:**

The influence of the COMT val158/108met polymorphism on non-motor symptoms in PD, particularly cognitive functions, has been studied but little is known about its effect on motor symptoms. Here, we hypothesized that COMT activity might modulate the age at onset (AAO) of motor symptoms in PD by modifying the bioavailability of the remaining endogenous dopamine in the striatum. Using the COMT val158/108met polymorphism as a surrogate maker of enzyme activity, we performed a two-step association study in 1067 patients and controls of European origin (French) from four independent samples.
Patients and Methods

French samples

A total number of 1,067 patients and 2,061 controls were included at the Paris site. PD subjects were recruited through the French network for the study of Parkinson's disease genetics (PDG) associating 15 university hospitals across France. Definite and probable PD were defined according to the United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) (Hughes et al, J Neurol Neurosurg Psychiatry 1992;55:181-184). Most (>80%) of the PD cases fulfilled the criteria for definite PD. All patients were of European origin, mostly French (>85%). A positive family history was found in 48% of the PD patients. The healthy controls of the French sample came from either the French Three-City (3C) cohort (n = 1933) or the PDG network (n = 128). The 3C cohort was assembled for a population-based, prospective (4-year follow-up) study of the relationship between vascular factors and dementia in three French cities: Bordeaux (Southwest France), Dijon (Central Eastern France) and Montpellier (Southeast France) (Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology 2003;22:316-325). The participants, non-institutionalized subjects over 65 years of age, were randomly selected from the electoral rolls of each city. Patients with Alzheimer's disease or other types of dementia and individuals for whom information on their dementia status during the 4-year follow-up was missing were excluded. The control subjects, randomly selected from among the participants, were matched for gender with PD patients.

Age at onset (AAO) definition

AAO was systematically determined at the time of inclusion by a retrospective interview. Disease onset was defined as the first PD-related motor symptom (akinesia, tremor or rigidity) experienced by the patient.

Genotyping

Genomic DNA was extracted by standard methods from venous blood samples. The COMT polymorphism G158A (SNP rs4680) was analyzed by an allelic discrimination Taqman assay (ABI PRISM 7900 sequence detection system) (Applied Biosystems, Forster City, USA) that uses the 50-nuclease activity of Taq DNA polymerase to detect
a fluorescent reporter signal generated after the polymerase chain reaction. Two controls with each of the $COMT^{HH}$, $COMT^{HL}$ and $COMT^{LL}$ genotypes were analyzed concomitantly with each patient.

Statistical analysis

Descriptive statistics used numbers and percentages as qualitative variables and means and standard deviations as quantitative variables. Relationships among qualitative variables were tested using chi square tests and relationships between age and quantitative variables were tested using ANOVAs followed by Tukey tests for pair-wise comparisons. AAO was analyzed using the survival data methodology; i.e., the survival distributions of AAO were estimated using the Kaplan-Meier method, univariate relationships between AAO and qualitative variables were tested using log-rank tests, univariate relationships between AAO and quantitative variables were testes using univariate Cox model, and relationships between AAO and several variables were tested using multivariable Cox models. Genotypes were coded as the number of "L" alleles; they were considered as quantitative variables in the codominant models and were coded as binary variables in the dominant model. Hardy-Weinberg equilibrium was tested using chi square tests in each sample. All tests were two-sided, with a p-value of 0.05 considered as significant. Computations were performed using the SAS V9 statistical package.

The study was carried out in accordance with the Declaration of Helsinki and the rules for clinical good practice. All participants gave their informed consent. The local Ethical Committees approved the study.

Results

Characteristics of patients and controls

A total of 3,128 subjects were available (1,067 PD patients and 2,061 controls). Subjects were genotyped for the rs4680 polymorphism. The characteristics of the subjects are summarized in Table 1.
Table 1: Clinical characteristics and distribution of the genotypes.

<table>
<thead>
<tr>
<th></th>
<th>French</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paris</td>
</tr>
<tr>
<td>Cases</td>
<td>1067</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>1.4</td>
</tr>
<tr>
<td>Age mean ±SD (n)</td>
<td>57.1 (±13.4) (1065*)</td>
</tr>
<tr>
<td>AAO mean ±SD (n)</td>
<td>48.39 (±13.16) (1031*)</td>
</tr>
<tr>
<td>Familial PD (%)</td>
<td>48</td>
</tr>
<tr>
<td>HH, n (%)</td>
<td>274 (26)</td>
</tr>
<tr>
<td>HL, n (%)</td>
<td>539 (50)</td>
</tr>
<tr>
<td>LL, n (%)</td>
<td>254 (24)</td>
</tr>
<tr>
<td>Controls</td>
<td>2061</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>1.3</td>
</tr>
<tr>
<td>Age mean ±SD (n)</td>
<td>73.7 (±5.4)</td>
</tr>
<tr>
<td>HH, n (%)</td>
<td>553 (27)</td>
</tr>
<tr>
<td>HL, n (%)</td>
<td>1057 (51)</td>
</tr>
<tr>
<td>LL, n (%)</td>
<td>451 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>3128</td>
</tr>
</tbody>
</table>

AAO = Age at onset; M = male; F = female; I+ = positive family history for Parkinson disease; SD: Standard deviation; *Number of subjects for whom age or AAO of PD disease were available; HH: homozygous for the G allele; HL: heterozygous AG; LL: homozygous for the A allele.

The SNP distribution was in Hardy-Weinberg equilibrium (HWE) in the patients and controls of each cohort (data not shown). The mean AAO is of 48.4 +/- 13.2 years for the French cohort (Table 1).

Age at onset and COMT polymorphism

The AAO was 48 (± 13), 48 (+ 13), and 50 (± 13) years for PD patients with COMT<sup>H</sup>H, COMT<sup>H</sup>L and COMT<sup>L</sup>L genotypes, respectively (Table 2).

Table 2: Genotype of the SNP rs4680 compared to age at onset of PD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Paris (n = 1031*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAO (SD)</td>
</tr>
<tr>
<td>HH</td>
<td>47.97 (±13.26)</td>
</tr>
<tr>
<td>HL</td>
<td>47.77 (±13.07)</td>
</tr>
<tr>
<td>LL</td>
<td>50.14 (±13.14)</td>
</tr>
</tbody>
</table>

AAO: age at onset of PD in years, values are means (+/- SD); HH: homozygous for the G allele; HL: heterozygous AG; LL: homozygous for the A allele; SD: Standard deviation. *Age at onset was not available for all patients
There was a significant association between the AAO and the genotype assuming a dominant model \((COMT^{III}/COMT^{III} \text{ versus } COMT^{LL}) [P = 0.02]\) (Figure 1; Table 3).

**Table 3:** Effect of genotype on AAO assuming a dominant model in the hypothesis generation and verification samples.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hypothesis generation (Paris)</th>
<th>Median (CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH/HL</td>
<td></td>
<td>48 (47-49)</td>
<td>0.02</td>
</tr>
<tr>
<td>LL</td>
<td></td>
<td>50 (47-52)</td>
<td></td>
</tr>
</tbody>
</table>

Median in years; CI = 95% confidence interval of the median; [III]: homozygous for the G allele; II: heterozygous AG; LL: homozygous for the A allele

The sample showed a sex ratio of 1.4 (male/female) and a high proportion of PD patients with a positive family history (48%) (Table 1). We adjusted therefore for gender and family history that did not change the significant result for the dominant model (data not shown). Finally, combined analysis of the patients showed a significant association between AAO and COMT genotype with the dominant model \((p = 0.02)\) and a trend toward association with the codominant model \((p = 0.07)\).

**Discussion**

We show that the \(rs4680\) polymorphism is a genetic modifier of the AAO in idiopathic PD patients: patients with high and intermediate activity genotypes \((COMT^{III}/COMT^{III})\) have an earlier age at PD onset than patients with low activity genotype \((COMT^{LL})\).

We have confirmed in a larger cohort that the COMT polymorphism is associated with the age at onset of Parkinson’s disease. In this cohort, the rs4680 (COMT Vall58Met polymorphism) was genotyped in a total of 16,609 subjects from five independent cohorts of European and North American origin (5886 patients with PD and 10 723 healthy controls). The multivariate found that the H allele had a significant effect on the age at onset with a younger AAO in patients with the H/H (57.1+13.9, \(p=0.03\)) as compared to the L/L genotypes (58.3+13.5) (see Table 4 below). The difference was
greater in men (1.9 years between H/H and L/L, p=0.007) than in women (0.2 years, p=0.81).

**Table 4:** Age at onset in PD patients according to COMT Val58Met genotype

<table>
<thead>
<tr>
<th></th>
<th>L/L</th>
<th>H/L</th>
<th>H/H</th>
<th>Univariate p-value</th>
<th>Multivariate p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>58.3 ± 13.5</td>
<td>57.4 ± 13.9</td>
<td>57.1 ± 13.9</td>
<td>0.041</td>
<td>0.026*</td>
</tr>
<tr>
<td>Men</td>
<td>57.9+ 13.6</td>
<td>57.1+ 13.6</td>
<td>56.0+ 14.1</td>
<td>0.013</td>
<td>0.007**</td>
</tr>
<tr>
<td>Women</td>
<td>58.8± 13.3</td>
<td>57.7± 14.3</td>
<td>58.6± 13.4</td>
<td>0.29</td>
<td>0.81**</td>
</tr>
</tbody>
</table>

H/H: homozygous for the H allele; H/L: heterozygous; L/L: homozygous for the L allele.

* Multivariate model including cohorts and gender as cofactors.

** Multivariate model including cohorts as cofactor.

**Example 2:**

This example describes a proof-of-concept pharmacogenetic clinical trial, whose aim was to demonstrate that tolcapone is effective in early PD patients carrying the high COMT activity genotype (COMT\textsuperscript{HH}).

The primary objective of this clinical trial was thus to demonstrate the efficacy and safety of tolcapone compared to placebo on motor symptoms in early PD patients carrying the high COMT activity genotype (COMT\textsuperscript{HH}). Secondary objectives were the following: (i) to determine the efficacy and safety of tolcapone compared to placebo on motor symptoms in early PD patients carrying the other COMT genotypes (COMT\textsuperscript{HL} and COMT\textsuperscript{LL}); (ii) to determine the efficacy and safety of tolcapone compared to placebo on motor symptoms in the whole population, (iii) to determine the efficacy and safety of tolcapone compared to placebo on motor symptoms in early PD patients according to genotype (genotype*treatment interaction); (iv) to compare the dopamine denervation in early PD patients according to the COMT genotype; (v) to determine the response to tolcapone adjusted on dopamine denervation; and (vi) to determine the response to tolcapone on cognitive function according to the COMT genotype.
Design and interventions
A multicenter, double blinded, randomized, parallel, placebo controlled, clinical trial was performed for testing a treatment with tolcapone at 300 mg/day during 3 months in early PD patients. Pharmacogenetic analysis were performed.

Patients
The clinical trials included 80 patients: 24 patients recived placebo (8 COMT^{HH}, 8 COMT^{HL}, 8 COMT^{LL}), 36 patients recived tolcapone (12 COMT^{TM}, 12 COMT^{TM}, 12 COMT^{LL}) and 20 COMT^{HL} patients were not randomized. The randomization was thus of 2:3 (2 placebo : 3 tolcapone).
The number of patient had been determined using Statistica software, and based on the result of the rasagiline trial (Stern, Movement disorders, 2004, 19(8):916-923), pharmacokinetic study of COMT according to genotype, and polymorphism distribution of COMT in caucasien population.

Inclusion criteria
Patients were men or women aged from 35 to 75, wherein PD was diagnosed since less than 3 years (PD defined as probable or possible on the UKPDS Brain Bank criteria). Patients must be able to stay 3 months with only the study drug as antiparkinsonian therapy. All included patients signed a written informed consent.

Exclusion criteria
Patients with at least one of the following criteria were excluded from the clinical trial: (i) patients for which a contraindication or intolerance to tolcapone was detected; (ii) patients with abnormal liver function or current liver disease; (iii) patients with severe renal failure with creatinine clearance < 30ml/min; (iv) patients with current treatment or previous treatment unless stopped since more than 3 months with any antiparkinsonian drugs including MAO inhibitors, selective or non-selective, dopamine agonist, levodopa and anticholinergic drugs; (v) patients diagnosed with an atypical parkinsonian syndrome or with a parkinsonian syndrome secondary to neuroleptic treatment; (vi) pregnancy and/or lactation; (vii) patients unable to sign informed consent or (viii) clinical dementia or MMS < 24.
Interventions

At the Screening visit, a blood sample was collected that was used to determine COMT genotype status: results determined the eligibility of the patient to be randomized. The target ratio for the COMT™, COMT™ and COMT™ was 1:1:1 respectively.

Treatment

Patients received 100 mg of Tolcapone or of placebo three times a day during 3 months.

Visits

8 visits were scheduled: Screening visit (Day - 30 (D-30), +/- 8 days), followed by an inclusion visit at day 0 (D0); 4 safety visits at days 15, 45, 60 and 75; a follow up visit at day 30 and a "end of study" visit at day 90. Content of each visit is shown in Table 5.

<table>
<thead>
<tr>
<th>Design</th>
<th>Screening visit</th>
<th>Inclusion</th>
<th>Tolcapone versus placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calendar</td>
<td>D-30+/8</td>
<td>D0</td>
<td>D15 D30 D45 D60 D75 D90</td>
</tr>
<tr>
<td>Visit</td>
<td>x</td>
<td>x</td>
<td>x x x x x x</td>
</tr>
<tr>
<td>Informed consent</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Inclusions/Exclusions criteria</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Randomization</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS-UPDRS</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Neuropsychological examination</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>PDQ36</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Plasmatic assay of COMT activity (T0 and T120 min)</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Genetic blood sample</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver function blood test</td>
<td>x</td>
<td>x x x x x x</td>
<td></td>
</tr>
<tr>
<td>Kidney function blood test</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observance</td>
<td>x x x x x x x x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>123I-FP-CIT</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>x x x x x x x x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5

Endpoints

Primary endpoint:
Change in total MDS-UPDRS between baseline and D90 was determined in the tolcapone and the placebo group and between genotypes (interaction genotype*treatment)

Secondary endpoints:
Impact of the treatment was assessed on (i) dopamine denervation on $^{123}$I-FP-CIT (SPECT), (ii) MDS-UPDRS subscores (I, II and III), (iii) cognitive function, (iv) PDQ-36, (v) COMT activity.

Statistical analyzes
Statistical analyzes were performed using Statistica, PAS and R softwares. Non-parametric analysis was used to compare quantitative traits (Kruskal Wallis Test) or qualitative traits (Mann Whitney Test). Determination of adjustment factors and predictive models were carried out by multiple regression analysis.

Example 3:
We investigated the effect of a COMT inhibitor, tolcapone, on the development of motor symptoms in an experimental model of chronic dopamine denervation in mice. The model used is a dose ascending treatment with MPTP as described by Goldberger and colleagues (Goldberg et al., Neuroscience 2011, 180:256-271).

Briefly, mice are administered a daily (5 d/wk) dose of l-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that increases weekly over the course of 4 weeks (4 mg/kg, 8 mg/kg, 16 mg/kg and 32 mg/kg). Behavioral assessment is performed each week. In this model, it has been shown that the chronic dopamine denervation correlated with progressive motor impairment assessed by Free-standing rears (FSR). Impairment of motor function occurs around 8 mg/kg and progress until 32 mg/kg.

We compared 2 groups of mice using this model to test the effect of the COMT inhibitor tolcapone on motor dysfunction occurrence:

- MPTP + tolcapone 10mg/kg i.p. daily,
Preliminary results are presented on Figure 2. In this experiment, mice treated with MPTP+tolcapone had higher FSR compared to mice treated with MPTP+vehicle at the dose of 4, 8 and 16mg/kg. The repeated measure ANOVA analysis showed a significant time*MPTP*tolcapone effect (p=0.006).

These results thus support the hypothesis that an inhibitor of the COMT enzyme administered at the beginning of the dopamine denervation may delay the occurrence of motor symptoms.
CLAIMS

1. A composition comprising an inhibitor of the COMT enzyme for use in the treatment of a Neurodegenerative Disease with Parkinsonian Syndromes in a subject having a COMT$^{HH}$ allele, wherein said composition does not comprise L-Dopa.

2. The composition for use according to claim 1, wherein the inhibitor of COMT is an inhibitor of COMT activity.

3. The composition for use according to claim 2, wherein said inhibitor of COMT activity is selected from the group comprising tolcapone, nitecapone, 2-(3,4-dihydroxy-2-nitrophenyl)vinyl)ketone, entacapone, dihydroxynitrobenzaldehyde, 6-nитронорадrenaline, dinitrocatechol, 1,2-dihydroxyl-3-hydroxypyridine-4-one; vinylphenylketone; CGP 28014; 1,2-dimethyl-3-hydroxypyridine-4-one, preferably said inhibitor of COMT activity is entacapone or tolcapone.

4. The composition for use according to claim 1, wherein the inhibitor of COMT is an inhibitor of the expression of the gene encoding the COMT enzyme.

5. The composition for use according to claim 4, wherein said inhibitor of the expression encoding the COMT enzyme is selected from the group comprising siRNAs, shRNAs, antisense oligonucleotide, ribozymes or aptamers of COMT.

6. The composition for use according to anyone of claims 1 to 5, wherein the patient is COMT$^{HH}$ or COMT$^{A}$. 

7. The composition for use according to anyone of claims 1 to 6, wherein said Neurodegenerative Disease with Parkinsonian Syndromes is Parkinson disease, progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration or Lewy body dementia.

8. The composition for use according to anyone of claims 1 to 7, wherein motor symptoms related to said Neurodegenerative Disease with Parkinsonian Syndromes are to be treated.
9. The composition for use according to anyone of claims 8, wherein said motor symptom to be treated is selected in the group comprising tremor at rest; akinesia and rigidity, such as, for example, slowness of movements, anemia, micrographia, loss of arm swing, difficulties in walking, sensation of stiffness; joint pain, dystonia, swallowing disorders, abnormal tiredness, trembling sensation, bradykinesia, action tremor, tremors, dysarthria, dysautonomia, dysphagia, dystonia, eye apraxia, limb apraxia, myoclonus, oculomotor tremors and night tremor.

10. The composition for use according to anyone of claims 1 to 9, wherein the subject is at risk of developing a Neurodegenerative Disease with Parkinsonian Syndromes.

11. The composition for use according to anyone of claims 1 to 10, wherein the subject is diagnosed with a Neurodegenerative Disease with Parkinsonian Syndromes.
FIG. 1
FIG. 2
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)
      - on paper
      - in electronic form

   b. (time)
      - in the international application as filed
      - together with the international application in electronic form
      - subsequently to this Authority for the purpose of search

2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
**INTERNATIONAL SEARCH REPORT**

**International application No**

**PCT/EP2013/055972**

### A. CLASSIFICATION OF SUBJECT MATTER

|------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|

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)
  - A61K

- 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)
  - EPO-Internal, WPI Data, BIOSIS, EMBASE, SCISEARCH, CHEM ABS Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

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

*"X" 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

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*"A" document member of the same patent family

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

7 May 2013

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

15/05/2013

**Name and mailing address of the ISA**

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NL - 2280 HV Rijswijk
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Fax. (+31-70) 340-3016

**Authorized officer**

Cielen, Else
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