A Gene Therapy Project for Lesch-Nyhan disease

Background on current research at iStem

For a few years now, three research teams at iStem have been working complementary to each other on Lesch-Nyhan disease.

> *Reminder: iStem (www.istem.eu) is the largest French research laboratory dedicated to developing human pluripotent stem cells. It is a collaboration of the French National Institute for Health and Medical Research - INSERM - and the French Association against Myopathies - AFM-Telethon -).*

Starting with skin biopsies originating from four patients with Lesch-Nyhan disease, these teams succeeded in creating pluripotent stem cells (iPSC) and grew them into cortical neurons (neurons involved in cognitive and intellectual functions).

> *Definitions: Induced pluripotent stem cells (iPSC) can in principle develop into any other type of human cell, including various types of neurons in the brain. However, the right "recipe" must be found for each cell type.*

The iStem teams are now attempting to develop the iPSC into dopaminergic neurons. These neurons synthesize dopamine, which is used as a neurotransmitter. It is known that dysfunction of these neurons is a cause of motor activity problems in Parkinson's disease, in Lesch-Nyhan disease, and also in other diseases with abnormal movements. The dopaminergic system is also involved in the development of self-injurious behaviour in Lesch-Nyhan disease and in some forms of autisms.

One of the goals of this work on the iPSC of Lesch-Nyhan patients is to understand how absence of the HRPT gene, and thus the enzyme of the same name, affects the development of neurons in the brain and causes the neurological symptoms of the disease (such as dystonia and self-injurious behaviour). Indeed, the link between lack of HPRT and the absence or failure of dopaminergic neurons is still a mystery (whereas the link between HPRT, uric acid and the kidney problems in this disease is already well understood).

The third iStem team works on a high throughput-screening project to test 20,000 molecules *in vitro* in order to find any that could potentially compensate for the absence of HPRT in cells of LND patients.

In parallel to this work, iStem also conducted preliminary work on possible approaches for gene therapy.

The Gene Therapy Project

On May 12th 2014, iStem announced to the representatives of the French Association *Lesch-Nyhan Action* (www.lesch-nyhan-action.org) that following the positive results of

the initial tests, they were beginning a preclinical gene therapy study for Lesch-Nyhan disease.

Definitions:

- *The principle of gene therapy is to inject a "vector" (a modified, non-harmful virus) that will bring to certain cells a corrected version of one or more genes. These corrected genes will allow the cells to create enzymes that were missing.*
- *During a preclinical study, the therapy is tested on animals in order to validate the effect of the treatment, evaluate its behaviour and determine toxicity (highest doses, side effects, etc.).*
- *Clinical trials on humans take place later on. These tests on volunteers aim to confirm the effects of the treatment and identify any adverse effects, in order to apply for marketing authorisation.*

The goal of this gene therapy in the case of Lesch-Nyhan disease is to restore dopamine levels in the brain, so that the symptoms of the neurological defect are reduced.

This project is coordinated by Alexandra Benchoua and involves iStem, the National Veterinary School of Nantes (www.oniris-nantes.fr) and a small UK biotechnology company, already involved in gene therapy for another disease with abnormal movements.

The Partner in Nantes is the Centre for Research and Preclinical Investigation (C.R.I.P.). It is a centre specialized in the preclinical validation on animals of gene therapies. The centre is partly funded by the AFM. This is where all the manipulations on animals will take place.

The principle of this project is to test on Lesch-Nyhan disease a therapy that has already been tested with some success in patients suffering from a different disease with abnormal movements (stabilization of dopamine levels and disappearance of dyskinesia).

A major benefit of this project is that the vector and the therapy itself have already been validated for another disease. Thus, it has only to be tested in the specific case of Lesch-Nyhan disease (first on animals and subsequently on humans). This is a huge timesaver.

Although there is hope to launch a clinical trial in the coming years, it is difficult to announce a timetable today, because there are still many uncertainties. First, a lot depends on what the tests on animals will show. Then, it is always possible that health authorities require testing on larger animals (dogs or monkeys, for example) before moving on to humans.

How does it work?

In Parkinson's and Lesch-Nyhan diseases, and also in some other disorders with abnormal movements, dopaminergic neurons are absent or deficient (for various reasons specific to each disease). As these are the neurons that make dopamine,

dopamine levels in the brain are very low, and this causes various motor problems that are observed in these diseases (and could possibly be one of the causes of self-injurious behaviour in Lesch-Nyhan disease).

The principle of the gene therapy that will be tested here is to use a vector (modified virus) that introduces in some non-dopaminergic neurons genes that enable them to produce dopamine. This should restore levels of dopamine in the brain, and hopefully improve the neurological symptoms of the disease.

This is therefore not a treatment for deficient dopaminergic neurons, but a way to make other neurons take over their functions.

Remarks:

- *Injection of the vector into the brain is performed by stereotaxy (neurosurgical technique to reach areas of the brain with precision). This is the same method used to take biopsies or apply electrodes in the case of deep brain stimulation. It is also the method used by Lysogene for their clinical trial on Sanfilippo disease (www.lysogene.com).*
- *This injection is done only once (once the genes are introduced, they continue to function by themselves). It is therefore a treatment done in one single "take".*
- *The problem is that once the vector is in place, you cannot stop the treatment (unlike a drug that can be stopped if it is not working properly). This is why animal testing is very important in order to understand all the consequences of the treatment.*

In a first step, iStem tested this vector in Petri dishes on non-dopaminergic neurons they created from normal iPSC and from Lesch-Nyhan iPSC (i.e. with or without HPRT). They were thus able to check that in all cases, irrespective of the presence or absence of HPRT, the enzymes were created, and dopamine was synthesized. These are the *in vitro* tests which gave the green light to move on to animal testing.

For the preclinical study, animal models had to be found on which to perform the essays.

Transgenic mice without the HPRT gene are a "gene" model of Lesch-Nyhan disease that is commonly used. However, although these mice do not have the HPRT gene (or the corresponding enzyme), they do not present symptoms of the disease (no self-injurious behaviour, no dystonia). Thus, these are not necessarily good models for the preclinical testing suggested by iStem. In particular, imaging or autopsies on these mice showed only a small reduction of dopamine, whereas dopamine loss in humans with Lesch-Nyhan disease is much higher.

The idea of iStem is to use another animal model of the disease. These are rats, in which the genetic code is not changed, but dopaminergic neurons are suppressed by injection of a certain drug, three days after birth. They grow up with no (or very little) dopamine, such as Lesch-Nyhan children. The advantage is that once the rats are teenagers or adults, they do present similar symptoms of Lesch-Nyhan disease (motor problems and self-injurious behaviour). It is therefore not a "gene" model but a "phenotypic" model of Lesch-Nyhan disease.

These rats are already recognized and validated by the authorities as models for Lesch-Nyhan disease. These same rats were also used for preclinical testing of Ecopipam (Dr Richard Chipkin, from Psyadon Pharmaceuticals laboratories).

During the study, iStem will inject the vector in rats of various ages and follow the outcome over several months. This will allow an investigation of the effects of the treatment according to the age of the patient and the duration. Then, the rats will be sacrificed and autopsied to measure the levels of dopamine produced (this measurement is difficult to perform by imaging only, because the animals are very small).

The pilot manipulation will take place in September/October 2014 on a reduced number of animals. Then it will be done on larger groups, to test several possibilities (treatments at different ages, for example).

Other remarks

In addition to the preclinical study, it would be very useful to implement an imaging study (MRI, or better PET Scan) of young children. But this is difficult, especially the PET scan, as the patient must be under general anaesthesia and radioactive material is injected. This scan will most likely only be authorized by the ethical review board (committee for the protection of persons) in the context of a clinical trial (because the risk is outweighed by the potential benefits), but not before.

Moreover, for the imaging study as well as for a possible trial, a dedicated neurologist will be needed to participate in the project.

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French Association Lesch-Nyhan Action
www.lesch-nyhan-action.org