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Press information

Successful treatment of adrenoleukodystrophy by transplantation of stem cells carrying a new gene therapy vector.

Two children with a fatal brain disease, adrenoleukodystrophy (ALD), have been successfully treated by using a new gene therapy vector. More than two years after the start of treatment, the progression of their disease has been stopped, and no side effect has yet been observed. The results of this clinical trial carried out by French research teams associating Inserm, AP-HP (Paris Public Hospitals) and Paris-Descartes University have just been published in the review *Science* dated 6 November 2009. This first concrete success confirms the hopes that HIV-derived lentivirus gene therapy vectors have therapeutic applications.

ALD is a rare genetic disease in the leukodystrophy group. In its most common and most serious form, it leads to the destruction of brain myelin of both children and adults. Use of the treatment initiated in 1989 by Patrick Aubourg and Pierre Bougnères with Claude Griscelli (Necker Children's hospital) based on a bone marrow allograft is restricted by the lack of compatible donors and the risk of serious complications.

The new approach consists in grafting the patient's own bone marrow cells after treatment by gene therapy without the need for a donor, thus avoiding the complications of transplantation. Bone marrow stem cells are harvested, then corrected by transfer of a functional version of the defective gene using a gene therapy vector derived from modified and inactivated HIV virus. After this repair, the cells are reinjected in patients in the same way as a conventional graft; they return to the bone marrow and some, by a natural mechanism, migrate to the patient's brain where they have a corrective role.

This clinical trial is the culmination of research undertaken by Nathalie Cartier, Inserm Research Director and Patrick Aubourg, Professor of Paediatric Neurology at Saint-Vincent de Paul hospital, Paris-Descartes University for 16 years. *"The two treated patients are doing well: the progression of the disease stopped a few months after the autograft"*, explained Nathalie Cartier. The patients were transferred from the Endocrinology and Paediatric Neurology Department of Pierre Bougnères and Patrick Aubourg at Saint-Vincent de Paul hospital to the departments of Marina Cavazzana and Alain Fischer at Necker Children's Hospital for the treatment of cells with the gene therapy vector and the autograft. The clinical trial was carried out in the Endocrinology and Paediatric Neurology Department at Saint-Vincent de Paul Hospital.

An innovative analysis of the genomic events in the corrected cells was performed by the team of Christof Van Kalle (Deutsches Krebsforschungszentrum, Heidelberg, Germany). *“Even if we must remain cautious, this analysis shows that we have no specific reason to fear a harmful effect linked to the integration of the lentiviral vector in the genome”* continued Nathalie Cartier. *“This is the first time a serious brain disease is successfully treated by gene therapy, giving a new impetus for the treatment of human diseases by this type of approach”* added Patrick Aubourg.

A scientific success, supported by a network of partners

These results illustrate the success of the transfer of knowledge from fundamental research to clinical medicine.

This research was performed thanks to exemplary collaboration between Inserm and the Paris Public Hospitals*. The project received constant support from ELA (European Leukodystrophy Association), the leukodystrophy patients association and the AFM (French Muscular Dystrophy Association, that organizes the Telethon).

For Guy Alba, founder chairman of the association and father of a child with adrenoleukodystrophy (ALD): *“thanks to the action of children in schools and the mobilisation of numerous donors and partners led by Zinedine Zidane, ELA are the number one source of funding for ALD research both at national and international level. Without neglecting other avenues of research, we have supported gene therapy for ALD and the team of Prof. Aubourg. Today we are particularly proud of the results as this approach opens new prospects for the treatment of other more common diseases. ELA will increase its financial outlay to help all patients benefit from this discovery, while continuing to accompany families from day to day as it has done for 17 years.”*

For Laurence Tiennot-Herment, President of the AFM: *“For more than 20 years, thanks to Telethon donations, we have given our unstinted support to researchers, such as Prof. Patrick Aubourg, who are developing the medicine of tomorrow. After the first results in gene therapy of immunodeficiency disorders, we are convinced that this new success will pave the way for a decade of results for other patients and other diseases. The medical revolution is accelerating. Here is proof that all together, we can be stronger than everything!”*

The development of such projects involves many players. At each stage, from manufacture of the vector up to the validation of the absence of toxic effects in cells, a whole network of public/private partnerships has been woven by Patrick Aubourg, requiring attentive management of intellectual property and partenarial research contracts. This coordination has been ensured for three years by Cécile Tharaud and her team at Inserm Transfert, a subsidiary of Inserm.

Nathalie Cartier and Patrick Aubourg are now planning to extend the trial in France and in the United States.

* This trial jointly financed by Inserm and the Paris Public Hospitals was carried out within the scope of the Hospital Clinical Research Programs (PHRC) of the Ministry of Research and the Hospitalisation and Organisation of Care Directorate (DHOS).

About Inserm

Inserm is the only French public organisation entirely dedicated to biological and medical research and to public health. Inserm conducts multithematic research. It permits the study of all diseases from the most common to the rarest. Inserm covers the whole pathway from the research laboratory to the patient's bedside. Since being entrusted with the task of coordinating French biomedical research in January 2008, Inserm has pursued functional reform to achieve this objective by setting up of eight thematic institutes. www.inserm.fr

Within Inserm, the Public Health thematic institute has the task of facilitating and coordinating the activity of research teams developing studies devoted to **public health** or **clinical research**, whether these teams are derived from Inserm, CNRS, or any other major research or university organisation.

About the Assistance Publique-Hopitaux de Paris (Paris Public Hospitals)

The AP-HP, Ile de France Teaching Hospital Centre, groups together 37 hospitals or hospital groups and coordinates the conduct of clinical research within its constituent hospital institutions. AP-HP therefore forms the first research centre on human subjects in Europe, with a number of active clinical research projects including:

- 797 on-going institutional research projects, sponsored or managed by AP-HP (on 31/05/2009), including 500 clinical trials with institutional sponsorship by AP-HP;
- 770 industrially sponsored clinical trials;

i.e. nearly 2,000 on-going research projects for all sponsors together in 2008. In 2008, 30,875 patients were included in AP-HP projects including 17,251 patients enrolled in clinical trials sponsored by the AP-HP and a budget of €37M was devoted to funding AP-HP projects.

About Inserm Transfert

Inserm Transfert is a private subsidiary founded in 2001 to manage the whole process of valorisation and transfer to industry of the knowledge gained by Inserm research laboratories, from invention disclosure reports to industrial partnerships. Inserm Transfert also proposes its services for the setting up and management of European and international projects, monitoring of clinical and post-listing studies, call management to institutional or industrial projects. Lastly, it has seed funds dedicated to life sciences, Inserm Transfert Initiative. www.inserm-transfert.fr

About the Paris Descartes Medical School

Paris Descartes University is the largest and most important French university in the field of health. This University for the health and social sciences, trains students in 4 major disciplinary fields: health, science and technologies, social sciences, law and economic sciences. The health pole of Paris Descartes University is recognised throughout Europe and the whole world for the quality of its training and the excellence of its research. www.parisdescartes.fr

About ELA

Established in 1992 with the constant support of Prof. Aubourg, ELA draws together families concerned with leukodystrophy. Since its inception, ELA pursues the same objectives: to help and support patients and their families and fund research. ELA pays particular attention to those who may not be able to benefit from the progress of research. For the same reasons, ELA supports the development of similar structures in Europe.

ELA set up its research foundation in 2005 at the request and with the support of the Ministry for Research to spur research into discovering a treatment,. ELA devoted 30 million Euros to leukodystrophy with preferential support for innovative programs such as gene therapy of

ALD. The results of Prof. Aubourg form part of this initiative and are a real hope for all patients.

About the AFM

Since 1958, the AFM has brought united the efforts of patients and their parents to pitilessly combat neuromuscular diseases which are genetic diseases that kill muscle after muscle. Committed to research and helping patients, it acts independently with, as sole guide, the urgency with respect to the disease and the patient's interest. Thanks to the generosity and loyalty of the public during Telethon, its action benefits all rare genetic diseases and common diseases. AFM is a major player in French biomedical research stimulating innovation and spurring the development of innovative therapies: 30 diseases are today at the trial stage of treatment in humans thanks to Telethon donations, www.afm-telethon.fr

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Background information about gene therapy and cell therapy

Gene therapy and cell therapy are two complementary fields of biomedical research with similar therapeutic goals. The strategy of these two approaches is to repair a deficiency (genetic or cell) by introducing a healthy version of the element concerned. Genetic therapy may be defined as the introduction of genetic material (usually the DNA contained in an inactivated virus) into the patient's cells to treat a disease. Cell therapy, on the other hand, consists in the transplantation of whole cells into a patient to treat the disease (for example by bone-marrow transplantation or even blood transfusion). The transplantation of cells of a given organ produced from stem cells is a particularly promising technique for the future repair of organs and even the brain and spinal cord.

For certain diseases, such as adrenoleukodystrophy, or severe combined immunodeficiency (SCID), a combination of gene and cell therapies is used. The modification of the patient's cells by genetic material (gene therapy phase) is then made *ex-vivo*, on harvested cells. The cells are then retransplanted back in the patient (cell therapy phase). The same type of combination is also developed for the treatment of certain cancers by modifying the patient's lymphocytes by gene therapy.

Key dates in gene therapy

1980: Martin Cline (UCLA, USA), against NIH recommendations, attempts to treat two patients with beta-thalassaemia (genetic disease affecting haemoglobin synthesis) and triggers public debate about gene therapy.

1989: Steven Rosenberg (National Cancer Institute, Bethesda, USA) shows that the injection cells carrying a gene transferred by a retrovirus into patients is not toxic.

1990: William French Anderson and R. Michael Blaese (NIH, USA) treat two children with adenosine deaminase deficiency associated with severe combined immunodeficiency (ADA-SCID). They used a murine retrovirus derived from MLV (Moloney murine leukaemia virus) to correct their lymphocytes. In 1993, R. Michael Blaese and Donald Kohn used the same vector to treat cells derived from the patient's umbilical cord. However the FDA (agency responsible for drug safety in the USA) did not authorise the associated myeloablation (elimination of diseased bone marrow cells from the patient before reinjecting the corrected cells) and mixed results were obtained.

1999: Jim Wilson (University of Pennsylvania, USA): 17 patients with a fatal metabolic disease of the urea cycle (ornithine transcarbamylase deficiency) are treated by injecting adenovirus containing the therapeutic gene. Jesse Gelsinger, the 18th treated patient, died after the injection of too high a dose and non-compliance with the trial inclusion criteria.

2000: Provisional suspension of all gene therapy trials in the United States by the FDA.

2000: Alain Fischer and Marina Cavazanna-Calvo (Paris-Descartes University and Inserm, Paris, France) use a MLV-derived retrovirus to successfully treat the bone marrow cells of children with X-linked severe combined immunodeficiency (X-SCID). 20 "Bubble children" have since been treated in France and England (by Adrian Trasher).

2002: One of the treated X-SCID patients develops leukaemia following the integration of the therapeutic gene close to a gene involved in cancer. Five of the 20 children treated in France and England developed leukaemia. A single patient died from the complications of the leukaemia. Seventeen of these 20 treated patients are still alive and benefit from the effects of treatment.

2005: Start of French gene therapy trials in adrenoleukodystrophy (Nathalie Cartier and Patrick Aubourg, Paris-Descartes University and Inserm) and beta-thalassaemia (Philippe Leboulch, Paris XI University and Inserm) using a HIV-derived lentivirus.

2006: First results of a gene therapy trial in AIDS using a gene therapy vector derived from HIV itself to introduce a therapeutic gene into the lymphocytes of AIDS patients (Boro Dropulic, University of Pennsylvania, United States). No side effect related to the use of this new vector was observed.

2007: First encouraging results of an intracerebral gene therapy trial in Parkinson's disease with an AAV (adenovirus-associated) vector (Matthew During, Cornell University, United States). Other similar trials based on the intracerebral administration of AAV or equine lentiviral vector are currently on-going in the United States and in Europe.

2008: First encouraging results of gene therapy with an AAV vector in a severe hereditary disease of the retina (R. Ali, University College London, England). Other similar trials with AAV or equine lentiviral vectors are currently being performed in the United States and in Europe.

2009: Alessandro Aitu and Maria Roncarolo (San Raffaele scientific institute, Milan, Italy) confirm the long-term beneficial effects and absence of complications of gene therapy in 10 children with ADA-SCID.

On-going trials in France

Three clinical trials using gene therapy are currently being carried out in France for genetic diseases. They aim to treat adrenoleukodystrophy, beta-thalassaemia and a form of myopathy (Gamma-Sarcoglycanopathy). Other trials will soon start to combat immunodeficiencies (Wiskott-Aldrich, X-SCID), a serious form of pigmentary retinitis (RP65), Duchenne myopathy, Parkinson's disease, Sanfilippo disease and metachromatic leukodystrophy.

A few figures on clinical trials in gene therapy

Since 1989, 1537 trials in man have been carried out in the world.

- 64.6 % against cancers.
- 8.9% against cardiovascular diseases.
- 8.1% against monogenic diseases.

Source: <http://www.wiley.co.uk/genetherapy/clinical/>

Vectors used in gene therapy

Retroviruses:

Lentiviruses, derived from HIV and used for example for gene therapy of adrenoleukodystrophy and beta-thalassaemia may be distinguished from murine retroviruses, derived from MLV (Moloney murine leukaemia virus) used for example in severe immunodeficiencies. Retroviruses are composed of RNA which must be retrotranscribed into DNA after entry in the cell. These are called "integrating" vectors as the retrotranscribed DNA is inserted into the genome of the infected cell. Lentiviruses are much more effective than murine retroviruses for correcting haematopoietic stem cells.

Adenovirus-associated viruses (AAV):

These viruses which are non-pathogenic in the natural state, are increasingly used for gene therapy. They are not integrated in the patient's DNA but may only be used to transfer small genes. Different versions of these viruses make it possible to target specific types of cells (neurons, retinal cells, liver cells). However, these vectors cannot be used to insert therapeutic genes into non-dividing cells such as haematopoietic stem cells.

New approaches:

New gene therapy approaches are also under evaluation. These involve "repairing" the gene, by using for example "zinc finger" RNA molecules that act like "molecular scissors" making it possible to correct a defective gene or to introduce a therapeutic gene.

The possibility of transforming skin fibroblasts into stem cells (iPS) may make it possible to use stem cells much more widely for repair and generally for combined gene and cell therapy trials.

Overview of adrenoleukodystrophy

The leukodystrophies form a group of 23 different hereditary diseases characterised by a disturbance of myelin formation or maintenance in the brain and spinal cord. Myelin constitutes the “white matter” and envelopes nerve fibres (axons) like a plastic sheath around electric cables: it permits the rapid conduction of nerve impulses. Myelin is manufactured by a specialised cell called an “oligodendrocyte”. Other brain cells also play a role in the formation and maintenance (i.e. integrity) of myelin sheaths once these have formed.

Adrenoleukodystrophy is the most frequent leukodystrophy (1 per 20,000 births). It is one of the first hereditary diseases for which the gene was identified by the “positional cloning” technique in 1993.

The most devastating reaction is the very rapid destruction of myelin in the brain which affects boys between 5 and 12 years and adults around 30 years. In a few months, this destruction leads to the loss of all their intellectual, motor and sensory functions and rapid death. The only possible form of treatment of these terminal cerebral forms of adrenoleukodystrophy is bone-marrow transplantation provided that this is performed very early after the onset of the first myelin abnormalities detected by brain MRI. However, all the candidate patients for a bone-marrow transplant cannot be treated because of the lack of donors or compatible cord blood in banks (as the stem cells required may also be extracted from umbilical cord blood). In addition, bone-marrow transplantation has a high risk of failure (rejection, graft-versus-host reaction) and mortality (15-20% in the child and nearly 40% in adults). Hence the idea of taking the patient's own bone marrow cells and correcting them “genetically” with a gene therapy vector before reinjecting them back into the patient.

Another form of adrenoleukodystrophy is characterised by spinal cord lesions in adult men and more than 65% of women carriers of the disease (this form of adrenoleukodystrophy is called “adrenomyeloneuropathy”). Research is currently being performed to understand why certain patients develop a very severe form with cerebral lesions whereas others are only affected later at the level of the spinal cord. The involvement of the spinal cord in adrenomyeloneuropathy leads to paralysis of the 2 legs in a few years. As the risks of mortality after bone-marrow transplantation are much higher in adults than in children, this treatment is not proposed to patients with adrenomyeloneuropathy. A series of clinical and experimental findings suggest that it may be possible in the medium-term to extend gene therapy to male or female patients with adrenomyeloneuropathy.

Screening for adrenoleukodystrophy at birth is also under very serious consideration. A pilot screening program is currently underway in the United States and may soon be implemented in France and in other European countries. This screening will make it possible to treat all patients, both boys or girls, before an irreversible neurological lesion occurs and thereby eradicate this devastating disease.

The ELA association brings together all the families concerned by leukodystrophies, including adrenoleukodystrophy. Through its Foundation, it stimulates and actively funds leukodystrophy research at international level.

Patients with leukodystrophy are specifically managed in France in the Rare Disease Reference centre in Paris (Prof. P. Aubourg, Saint-Vincent de Paul Hospital) and at Clermont-Ferrand (Prof. O. Boespflug).

Presentation of unit U745

Inserm unit UMR745 “Genetics and biotherapies of degenerative and proliferative nervous system diseases” are located on the Paris-Descartes University campus in the Pharmaceutical and Biological Sciences Faculty of Pharmacy.

This research unit employs 22 persons including 8 researchers and lecturer-researchers, 5 research engineers/technicians, and 6 Post-docs and PhD students. The scientific strategy of the unit revolves around combating several serious genetic diseases of the nervous system, leading to severe disabilities and early mortality:

- Leukodystrophies (hereditary central nervous system demyelinating diseases);
- Type 1 neurofibromatosis;
- Alzheimer's disease.

To achieve this objective, the following are being developed in an integrated way:

- Therapeutic interventions centred around gene therapy (validation on relevant animal models, design of therapeutic vectors, preclinical and especially clinical implementation of these approaches in man) for two leukodystrophies (adrenoleukodystrophy, metachromatic leukodystrophy) and Alzheimer's disease;
- A phase III molecular screening platform to treat a devastating complication of type 1 neurofibromatosis: neurofibrosarcomas;
- Studies performed to find genetic variants influencing the phenotypic expression of leukodystrophies and type 1 neurofibromatosis.

Unit U745 maintains very close contacts with clinical departments at Saint-Vincent de Paul Hospital (paediatric neurology – Leukodystrophy Reference Centre) and Henri-Mondor hospital (dermatology, type 1 neurofibromatosis Reference centre) and collaborates in Europe and the United States with all the hospital and academic structures involved within leukodystrophy.

Part of the projects on leukodystrophies and Alzheimer's disease are developed in close cooperation with several international research centres (MIT in the United States, Max Planck Institutes in Germany, TIGET-HSR in Italy, AMC in the Netherlands, Ibdell in Spain).

Biographies of Nathalie Cartier and Patrick Aubourg

Dr Nathalie Cartier, paediatrician, is an Inserm Research Director and currently pilots the Biotherapies group in Inserm laboratory U745 directed by Patrick Aubourg.

After her internship in medicine in the Paris Hospitals, she worked as a post-doc at the Cochin Institute in the laboratory directed by Axel Kahn and developed the first models of transgenic animals required for research in oncogenesis. Then in 1993 she joined the laboratory of Pierre Bougnères (Inserm) to work with Patrick Aubourg on the gene involved in adrenoleukodystrophy and the development of a gene therapy strategy against this disease

Nathalie Cartier is vice-president of the French Cell and Gene Therapy Society. She plays an active role in national and European networks and committees aiming to develop and implement new therapeutic approaches in gene therapy. She also contributes to training a new generation of researchers in this field, *via* cycles of teaching at Paris-Descartes and Paris-Diderot universities.

For her career, she has been awarded the Academy of Medicine Prize, the Jean Valade Prize from the *Fondation de France* and the Thermo Biotherapy Prize.

Dr. Patrick Aubourg, paediatric and also adult neurologist, is Professor of Paediatrics and director of Inserm unit UMR745 at the Paris-Descartes University Faculty of Pharmaceutical and Biological Science. He also directs the department of Neuropaediatrics at *Saint-Vincent de Paul* Hospital in Paris.

In 1985, after his internship and registrarship in medicine at the Paris Hospitals, he worked as a postdoc in the laboratory of Hugo Moser at Johns-Hopkins University (USA). In 1987 he joined the Inserm laboratory of Pierre Bougnères and identified in 1993 the adrenoleukodystrophy gene with Jean-Louis Mandel.

Since 1987, Dr. Patrick Aubourg is particularly involved at national and international level in clinical and fundamental research with the objectives of developing and implementing new therapeutic approaches for neurodegenerative diseases of the child, in particular leukodystrophies

. Dr. Patrick Aubourg has made major contributions to the fight against these latter diseases through the development and action of the association ELA. He was chairman of the scientific committee of this association until 2008.

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