**The first gene therapy trial using HIV1-derived lentiviral vector to express a « therapeutic » gene in human hematoipoietic stem cells : preliminary results are encouraging**

Expression of « therapeutic » gene in blood cells (lymphocytes, red cells, granulocytes) or bone marrow derived cells (macrophages in brain, liver, lung, etc..) has major therapeutic interest in many human diseases, including in particular cancers (leukemia) and a large number of rare inherited diseases. Among these rare diseases are those for which the expression of the « therapeutic » gene must be achieved in specific blood cell population, for example, red cells for thalassemia and sickle cell disease that affect several hundreds of thousands of patients around the world, in lymphocytes or granulocytes for several inherited inmmunodeficiency disorders, and those for which the « therapeutic gene » must be expressed in brain macrophages (also called microglia), as is the case in a subset of inherited neurodegenerative diseases that affect the cerebral cortex, the white matter or both (Hurler disease, metachromatic leukodystrophy and X-linked adrenoleukodystrophy).

For nearly all these diseases, the expression of the « therapeutic » gene and therefore the « therapeutic » protein must be achieved for the entire life of the patient. Given the limited half-life of lymphocytes, red cells, granulocytes and macrophages, long-term expression can be obtained only if one succeeds in expressing the « therapeutic » gene in bone marrow cells that give continuously rise to lymphocytes, red cells, granulocytes and macrophages during the life of human, i .e, the so-called hematopoietic stem cells.

For all the inherited diseases mentioned above, this aim has been successfully achieved through a bone marrow transplantation procedure, also called allogenic hematopoietic stem cell transplantation. Allogenic bone marrow transplantation requires however that a matched-HLA donor (unrelated voluntary donor or cord blood) be found and this procedure remains associated with significant morbidity and mortality risks (failure of/incomplete grafting, graft versus host disease, chronic immune insufficiency). Transplanting the patient's own bone marrow cells into which the « therapeutic » gene has been introduced would circumvent the need for a donor, and eliminate many risks of bone marrow transplantation, in particular the risk of graft versus host disease.

Up to now, this gene therapy strategy has proved to be successful in only two very rare inherited forms of severe combined immunodeficiency disorders (SCID) : the adenosine desaminase (ADA) deficiency and the deficiency of the common γ chain (SCID-X1). The γ chain is common to five cytokine receptors, all of which are necessary for the development of T lymphocytes. In ADA deficiency and SCID-X1, the transfer of the « therapeutic » gene in hematopoietic stem cells of patients was achieved using a defective Moloney murine leukemia virus vector. This kind of gene therapy vector is poorly effective in transferring genes into non-dividing cells such as hematopoietic stem cells. In these 2 diseases, very few early hematopoietic progenitors were corrected initially, but clinical benefits (i.e repairing the immune system of patients) were obtained because, the few lymphocytes that originate from corrected hematopoietic progenitor cells expanded because of « selective advantage » that corrected/normal cells have over diseased cells. This property of « selective advantage » occurs in very few diseases and gene transfer using Moloney murine leukemia virus vector is therefore useless for most diseases.

With progresses made in the field of AIDS research, it has been possible to design a HIV1-derived gene therapy vector that is defective for replication (« non-infectious »), but that

keeps an essential property of HIV-1 virus : the potential to transfer DNA material and therefore the « therapeutic » gene into non-dividing cells.

The group of Patrick Aubourg and Nathalie Cartier in Paris (hospital Saint-Vincent de Paul, INSERM U745) reports the first attempt to transfer a « therapeutic » gene into hematopoietic stem cells in two patients with X-linked adrenoleukodystrophy (ALD). ALD is the most frequent form of leukodystrophy (a group of inherited disorders affecting the myelin within the central nervous system). Once symptoms have started in affected ALD boys, the disease progresses rapidly to a vegetative stage or death within 2-3 years. Patrick Aubourg showed in 1990 that allogenic bone marrow transplantation can arrest and even sometimes reverse the cerebral demyelination in ALD boys when the procedure is performed at an early stage, i.e before evident clinical symptoms occur. In ALD, the efficacy of allogenic bone marrow transplantation is mediated through the replacement of diseased brain macrophages of the patient by normal bone marrow-derived cells that penetrate into the brain and differentitate into normal brain macrophages.

In this first hematopoietic stem cell gene therapy trial with HIV1-derived lentivector (the sponsor of this trial is the french INSERM, Institut National de la Santé et de la Recherche Médicale), CD34+ cells (a sub-population of bone marrow cells that contains hematopoietic stem cells and which is commonly used to perform allogenic bone marrow transplantation in human) from 2 ALD patients who were candidates for bone marrow transplantation but who had no HLA-matched donor were genetically corrected ex vivo with a lentivector expressing the ALD gene (vector and assay reagents provided by Cell Genesys, Inc., South San Franciso, CA, USA). After all tests assessing the safety of manipulated cells had been performed, genetically corrected CD34+ cells were re-infused to the 2 patients after myeloablation (the same chemotherapy regimen is used for allogenic bone marrow transplantation). The first patient was treated 1 year ago, the second 6 months ago. The procedure occured without complications in the two patients who had hematological recovery 15 days after transplant and full immune reconstitution 1 year for the first treated patient and 6 months for the second treated patient after the re-infusion of the cells. Thus far, all tests evaluating the safety of the procedure, in particular the safety concerns for the use of HIV1-derived vector are negative. Importantly, biological tests show stable and high expression of the « therapeutic » protein and at the same percentage in all blood cells (lymphocytes, granulocytes, monocytes) deriving from bone marrow cells. These data indicate for the first time that in the absence of selective advantage, a high percentage of hematopoeitic cell progenitors with « self-renewal » (as true hematopoietic stem cells) were corrected giving rise to expression of therapeutic protein at high level in all hematological lineages. For ALD disease, the short-term evolution was similar to that observed in patients treated with conventional allogenic bone marrow transplantation. As for allogenic bone marrow transplantation, it will be necessary to wait up to 18-24 months after the transplant to determine if this gene therapy approach will be sufficienct to arrest the cerebral disease.

These data support the hopes that have been put in HIV-1 derived lentivector, i.e, their capacity to transfer « therapeutic » gene at high efficiency and for long life in non-dividing cells, including hematopoietic stem cells.

However these results are still preliminary and important pending questions need to get answered long term :

1/ first all the safety issues regarding the use of HIV1-derived vector and the risk of all

retrovirus vector to induce insertional mutagenesis (all retrovirus vectors integrate in or between genes in chromosomes and may activate nearby genes that play a role in oncogenesis)

2/ the real long-term (> 2years) stability of « therapeutic » gene expression in early hematopoietic progenitors with « self renewal ».

3/ whether the percentage of corrected cells achieved in this first trial will be sufficient to arrest the cerebral disease in ALD patients.

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