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PRECISION MEDICINE AND TARGETED THERAPIES:
REALITIES AND PERSPECTIVES

Gene Therapy: Clinical Achievements and Future Prospects

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Notes are linked to the references page.

FIRST STEPS IN THE DEVELOPMENT OF GENE THERAPY

Gene therapy, an old concept

The idea of using genetic material to treat certain diseases was born several decades ago. As early as the 1960s, it had been established that a viral genome could integrate into the cellular genome. In the late 1970s, cloning of genes became possible and pioneers such as Theodore Friedman and Richard Roblin were already imagining that modifying retroviruses to carry genetic information could correct genetic abnormalities. In 1969, Hurair Vasken Aposhian was stating in scientific congresses that, "if the role of a drug is to restore the function of a particular physiological process, then DNA should be considered the ultimate drug".

The 1980s-1990s: development of viral vectors and the first gene therapy trials

The development of viral vectors picked up speed thanks to a better understanding of the life cycle of retroviruses. The early 1980s saw the development of techniques to modify the retroviral genome by adding potentially therapeutic genes. In parallel, the concept of gene therapy by *in vivo* or *ex vivo* gene transfer into mammalian cells was a trending topic. Gene therapy gained momentum thanks to major progress in the field of stem cell biology and the manipulation of hematopoietic stem cells.

Thus, by the end of the 1980s, the foundations of the first clinical trials of gene therapy had been laid and the very first clinical gene transfer, involving insertion of a marker gene into the lymphocytes of cancer patients, was carried out in 1989 by Steven Rosenberg's group at the National Cancer Institute in Bethesda. The first therapeutic gene therapy trial took place in 1990 in Bethesda, led by Michael Blaese's group, for the treatment of severe combined immunodeficiency (SCID) due to adenosine deaminase deficiency.

In 1994, a group headed by Alain Fischer, Marina Cavazzana and Salima Hacein-Bey-Abina at Necker Hospital initiated a gene therapy trial involving gene transfer into hematopoietic stem cells for the treatment of X-linked SCID, known as SCID-X1[1]. This clinical trial provided proof-of-concept of the efficacy of gene transfer into patients' stem cells using a retroviral vector.

Since then, a large number of clinical trials in hereditary diseases, but also in cancer and chronic viral infections such as HIV, have been carried out. Most make use of viral vectors derived from a retrovirus, lentivirus, adenovirus or Adeno-Associated Virus (AAV) to carry a gene into the target cell. After transduction of target cells by integrative viral vector, the vector genetic material is inserted into the target cell genome and functional proteins are produced from the therapeutic gene, thereby restoring the cell to normal function.

DISEASES AFFECTING THE LYMPHOHEMATOPOIETIC SYSTEM

Hematopoietic stem cells quickly became a prime target for *ex vivo* gene transfer. In fact, it soon became clear that the stable integration of a therapeutic gene would guarantee a permanent correction of the disease thanks to the capacity of these cells for self-renewal.

Characteristics of SCID-X1

SCID-X1 occurs due to a very early block in the differentiation of T lymphocytes and NK (natural killer) cells. This block is caused by mutations in the gene encoding the gamma chain (γc) common to several cytokine receptors, including IL-7 and IL-15 which are involved in the development of these lymphocytes.

This lymphocyte deficiency is expressed clinically by recurrent, severe and lethal infections and by the need to keep young patients in sterile chambers (bubble babies). The standard treatment is allogenic bone marrow transplantation from a related donor with identical histocompatibility tissue antigens (HLA, for Human Leukocyte Antigen). Unfortunately, an HLA-identical match is uncommon and bone marrow transplantation from a haploidentical donor is associated with low 5-year survival rates.

First gene therapy trial for SCID-X1

SCID-X1 is an ideal model for the development of gene transfer therapy: there is no entirely satisfactory treatment for patients in the absence of an HLA-identical donor, and the genetic basis of the disease is known, T cell progenitors have a very high proliferation capacity, and correction of γc expression should confer a selective proliferation advantage.

Gene transfer consists of removing a sample of bone marrow from the young patient, introducing the therapeutic gene *ex vivo* into stem cell explants using an integrative vector, and then infusing the modified cells back into the patient whose immunity will thereby be restored. The first generation vector used in 1999 was derived from Moloney retrovirus (murine leukemia virus). Ten patients under one year of age were treated. Immune reconstitution was achieved in nine of the ten treated patients, but four of them went on to develop T-cell acute lymphoblastic leukemia (T-ALL) and one consequently died[1].

This first trial taught us that there is a positive correlation between the extent of T cell reconstitution and the size of the genetically modified graft[2]. In the patients who developed leukemia, chemotherapy did not eradicate the modified T cell progenitors and T lymphocyte counts were restored. It should be pointed out that the T cell repertoire and diversity were normal and comparable to that of the polyclonal population in a healthy individual.

Analysis of T-ALL cases

The retroviral vector expressing the γc chain complementary DNA did therefore restore immunity in the majority of patients, but at the cost of developing T-ALL in four of the nine patients, 31 to 68 months after gene therapy. Further analysis showed that the viral vector had integrated in or near proto-oncogenes, with a predilection for the proto-oncogenes LMO2 and CCND2, which are known to play a role in the development of T-ALL. We have shown that the mechanism of activation of these proto-oncogenes is related to their proximity to the insertion sites of the viral promoter[2,3].

Second gene therapy trial for SCID-X1

The first generation of viral vectors conserved the intact viral promoter, which is what caused its transactivating effect on oncogenes in proximity to the virus insertion site. It was clear that these vectors now had to be improved to enhance their safety. Accordingly, we assessed the efficacy and safety of a safer, self-inactivating (SIN) retroviral vector in which the enhancer sequences of the viral promoter were deleted.

We initiated a multicenter trial in France, England and the United States enrolling nine boys with SCID-X1[4]. All patients received bone marrow-derived CD34+ cells transduced with the SIN-γc vector. Eight patients had recovery of functional T cells leading to resolution of infections (see Fig. 1). Analysis of the insertion site profile revealed significantly fewer retroviral insertion sites within (or in proximity to) lymphoid proto-oncogenes as compared with the vector used in the first gene therapy trial for SCID-X1 (see Fig. 2). The patients have remained healthy and leukemia-free seven years after gene therapy.

Figure 1. Change in CD3+, CD4+ and CD8+ lymphocyte counts over time[4]

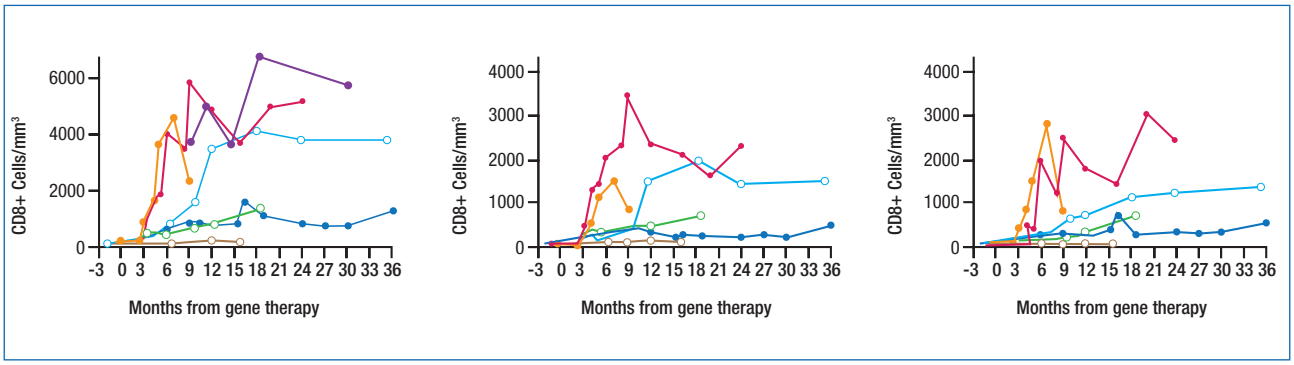
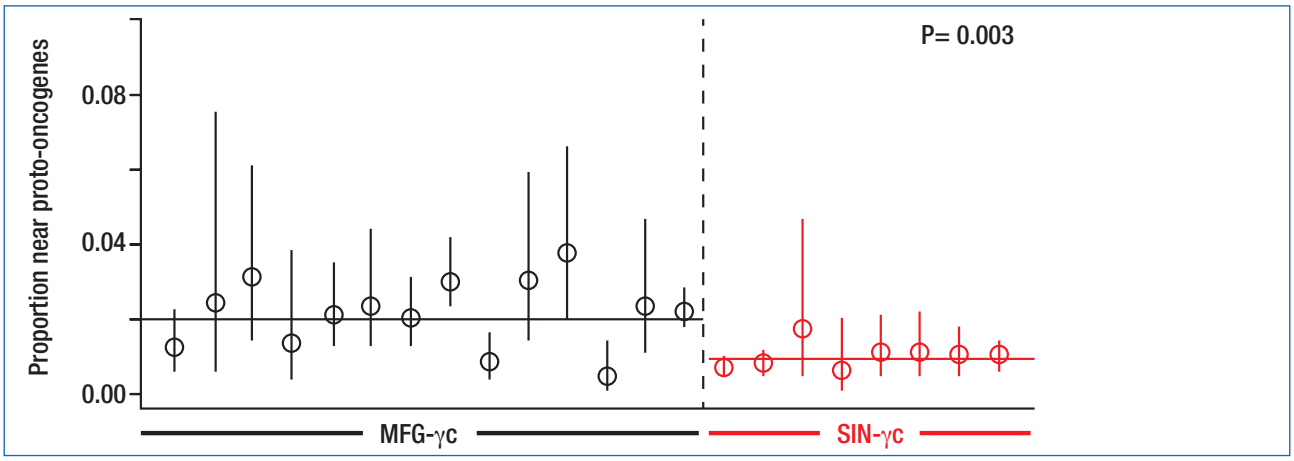


Figure 2. Comparison of frequencies of integration sites near proto-oncogenes[4]



Each point represents an individual patient who has undergone gene therapy. P = 0.003 is for the comparison between trial on a per-patient basis (each patient was analyzed as a single data point). MFG-γc: first generation Moloney murine leukemia vector expressing complementary DNA for γc; SIN-γc: viral-enhancer-deleted self-inactivating retroviral vector.

EXPANDING THE CLINICAL APPLICATIONS OF HEMATOPOIETIC STEM CELL GENE THERAPY

A large number of clinical trials conducted over the past two decades have made use of self-inactivating integrative retroviral vectors or, more recently, lentiviral vectors. The results have not always been encouraging, but we will cite the work of our Italian colleagues in Milan who have successfully conducted trials in the treatment of immune deficiencies, including adenosine deaminase deficiency[5]. Several groups, including ours at Necker Hospital in collaboration with Genethon, have studied Wiskott-Aldrich syndrome. This trial showed good clinical outcomes after WASP gene transfer into the hematopoietic cells of the patients[6,7]. Finally, several groups including ours at Necker Hospital have undertaken the treatment of beta-thalassemias by gene therapy, with very encouraging results.

CLINICAL APPLICATIONS OF LIVER-TARGETED GENE THERAPY

An ideal target for the production of therapeutic proteins

It has been suggested that after transduction, hepatocytes may be able to produce and release into the general circulation proteins capable, for example, of treating defects such as factor IX deficiency.

As hepatocytes are differentiated cells with a long lifespan, it was not necessary to select a strategy based on the use of an integrative vector. The choice turned towards an AAV-derived non-integrative vector. AAV viruses have the advantage of exhibiting tropism for liver and persist in hepatocytes as episomes. This family of vectors is widely used in *in vivo* gene therapy protocols targeting brain, retina, muscle and liver.

Gene therapy for hemophilia

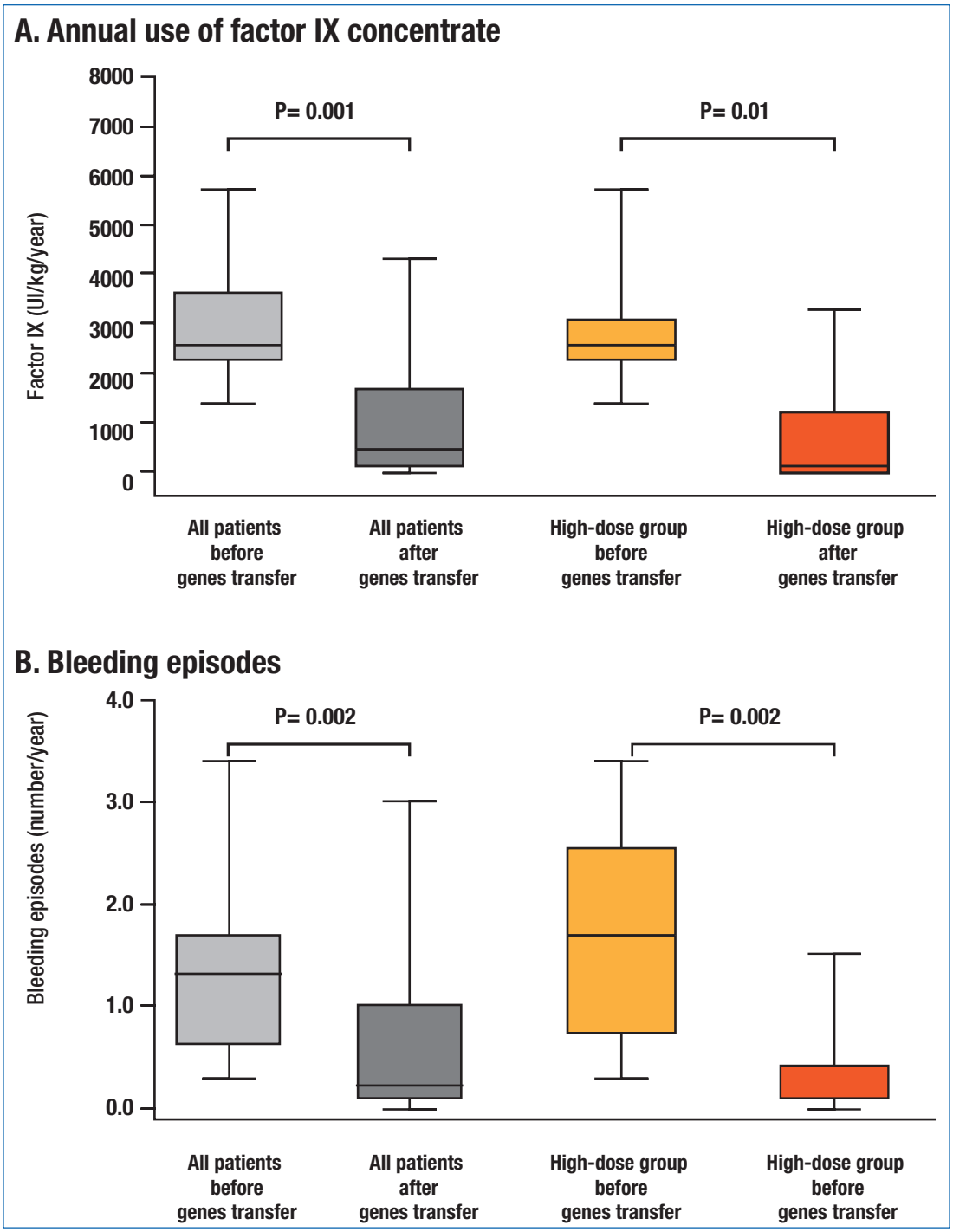
Hemophilia B is the second most common hereditary bleeding disorder. It is a potentially fatal disease characterized by frequent joint bleeds which lead to cartilage damage, fibrosis and loss of joint space. Currently, the treatment of hemophilia consists of administering recombinant or plasma-derived clotting factor concentrates.

Here again, clinical trials of gene therapy for hemophilia have a long history. The first trial in this indication was published in 2006[8]. Those investigators infused an AAV serotype 2 vector expressing human factor IX through the hepatic artery into seven patients. The intervention was well tolerated and factor IX levels increased very rapidly. However, therapeutic levels of factor IX expression were limited to around 8 weeks due to the destruction of transduced hepatocytes by the immune response targeting AAV capsid proteins.

A later study by Nathwani and Davidoff in London used another AAV serotype, AAV-8, with higher liver tropism than AAV-2 and with an improved design to increase the potential for transgene expression[9]. We know that immunogenicity is proportional to the injected dose of vector genomes, and therefore that the more effective the vector, the less the need to increase the dose, which probably contributed to the success of this trial. Furthermore, the investigators induced a transient immunosuppression by administering corticosteroids very early at trial initiation.

A single intravenous infusion of this vector in 10 patients with severe hemophilia B resulted in a dose-dependent increase in circulating factor IX to a level that was 1 to 6% of the normal value. In the high dose group, the factor IX level increased to a mean of 5.1%, which resulted in a more than 90% reduction in bleeding episodes and the use of prophylactic factor IX concentrate (see Fig. 3).

Figure 3. Effect of gene transfer on the administration of factor IX concentrate and the number of bleeding episodes before and after gene transfer[9]



Comparison of the mean amount of factor IX concentrate used (A) and the number of bleeding episodes (B) in the 10 study patients during the year preceding gene transfer and during the year following gene transfer.

CLINICAL APPLICATIONS OF GENE THERAPY IN CANCER

Gene therapy is considered an effective treatment for hereditary diseases but, to date, the largest number of gene transfer clinical trials has been in cancer, probably because of the gravity of the disease and/or the large number of affected patients.

Therapies based on the use of oncolytic viruses

The early gene therapy clinical trials in cancer made use, sometimes very effectively, of oncolytic viruses. Oncolytic viruses specifically induce the death of tumor cells. They are an emerging treatment option for many types of cancer and have recently been the focus of extensive research aiming to develop their therapeutic potential. The ultimate aim is to design a virus that can effectively replicate inside the host cell, specifically target and lyse tumor cells and induce robust, long-lasting tumor-specific immunity. There are a number of viruses that are either naturally tumor-selective or can be modified to specifically target and eliminate tumor cells[10].

Cancer immunotherapy: CAR-T cells

We must also draw attention to a major technological breakthrough that has occurred in recent years involving an immunotherapy based on infusion of autologous T cells modified to express a special, "chimeric" receptor (CAR: *Chimeric Antigen Receptor*), allowing them to specifically recognize tumor cells.

This therapy works by redirecting a patient's T cells to recognize a specific tumor antigen and destroy the cells that express it. In the majority of cases, the patient's T-cells are transduced with a lentivirus vector coding for a CAR targeting the CD19 cell surface antigen expressed in acute lymphoblastic leukemia. In practice, the patient's white blood cells are isolated, the autologous T cells are transduced to express a CAR receptor, and then re-infused into the patient. These T cells can then expand, "hunt down" and kill the cancer cells.

While the first CARs afforded only a limited response in clinical trials due to their limited persistence, the new generation CARs, equipped with additional costimulatory domains, have shown impressive results[11]. CAR-T cell therapy is thus recognized as a pioneering technology shown to be particularly effective in treating patients with refractory or recurrent leukemia or lymphoma. More than 200 CAR-T clinical trials have been implemented so far, most of which involve the treatment of B-cell malignancies using CD19-specific CARs. The first successes were published in 2011 by Carl June's group at the University of Pennsylvania[12, 13].

A growing number of studies are also investigating solid tumors, and new studies on cancer immunotherapy are currently exploring the potential of NK cells engineered to express a CAR of interest.

TECHNOLOGICAL DEVELOPMENTS IN GENE EDITING

In recent years, considerable progress has been made in other areas of biotechnology such as gene editing. CRISPR-Cas9, a bacterial RNA with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated with a nuclease system (Cas) is widely used to correct mutations in eukaryotic cells or to design T cells that can be used in cancer immunotherapy.

The first clinical trial using the CRISPR-Cas9 system was carried out in China in 2016 in patients with lung cancer[14]. Currently around twenty trials are in preparation, mostly in China.

It should be noted that the accuracy of this technology is a critical requirement and that the risk of "off-target" editing, i.e. modifying genes other than those targeted, raises concerns because of the potential for serious adverse events, including cancers. The CRISPR-Cas9 technique holds great promise, but effective distribution technology will be needed for large, high-performance applications with an accurate assessment of off-target toxicity.

CONCLUSION

Gene therapy began with the simple idea that replacing a defective gene with a functional copy could help cure a disease. The road has been long for this concept to become reality. Recent gene therapy trials have shown important benefits. These clinical outcomes, together with: 1) improvements in the viral vectors used for gene transfer; 2) implementation of the concept of cancer immunotherapy as a potent new therapeutic option for a growing number of cancer types; 3) development of gene editing techniques, have contributed to the revitalization of the gene therapy field.

The future clinical development of these cell therapies will be guaranteed by the creation of scalable technology platforms that: 1) promote different levels of pharmaceutical grade quality production; 2) adapt to the growing ability to target the sites of gene insertion. These new gene/cell therapy products hold forth the promise of safe and effective treatments for incurable diseases and will be accessible to the widest number of patients.

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